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Jnana Kaveri PG Centre, Chikka Aluvara, Kodagu District, Karnataka

Effect of chemical elicitors for improved quality of turmeric (*Curcuma longa* L.)

Ph.D. Final Synopsis

Submitted to Department of Biochemistry

Mangalore University

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOCHEMISTRY

By

Sivaranjani. R

No.MU/EXB/Ph.D./CR.173/ Biochem./2018-19/E.13

Under the Guidance of

Dr. T John Zachariah

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Kozhikode, Kerala

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Abbreviation:

- CHT - Chitosan
- PA - Phenylalanine
- SA - Salicylic acid
- Acc. - Accession
- HPLC - High Performance Liquid Chromatography
- PDA - Photo Diode Array
- GC-MS - Gas Chromatography – Mass Spectrometry
- LRI - Linear Retention Indices
- CAT - Catalase
- SOD - Superoxide Dismutase
- POD - Peroxidase
- PAL - Phenylalanine Ammonia Lyase
- PPO - Polyphenol Oxidase
- Chl a - Chlorophyll a
- Chl b - Chlorophyll b
- Total Chl - Total Chlorophyll
- BDMC - Bisdemethoxycurcumin
- DMC - Demethoxycurcumin
- TC - Total curcuminoids
- Pn - Photosynthetic rate
- E - Transpiration rate
- Gs - Stomatal conductance
- IISR - Indian Institute of Spices Research
- ICAR - Indian Council of Agricultural Research
- EO - Essential oil
- TPC - Total Phenolic content
- TFC - Total Flavonoid content
- DPPH - 2,2-diphenyl-1-picrylhydrazyl
- ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
- PLS-DA - Partial Least Square-Discriminant Analysis
- VIP - Variable Importance of Projection

- SD - Standard Deviation
- SE - Standard Error
- PCR - Polymerase chain Reaction
- RNA - Ribo nucleic acid
- cDNA - complementary Deoxyribo Nucleic Acid
- q RT-PCR - Quantitative Real Time Polymerase Chain Reaction
- EtBr - Ethidium Bromide
- mL - milliliter
- kg - Kilogram
- mg - milligram
- g - gram
- mM -millimolar
- DMRT - Duncan's Multiple Range Test
- VIP - Variable Importance in Projection
- PCA - Principal Component Analysis
- PLS-DA - Partial Least Square – Discriminant Analysis
- nm - nanometer
- μM - micromolar

CHAPTER 1

Introduction and Objectives

Turmeric (*Curcuma longa* L.) revered as "Golden Spice" is a rhizomatous crop belongs to Zingiberaceae family. The crop is native to tropical Southeast Asian region (Ferreira et al., 2013). India is the largest producer, consumer and exporter of this crop. The economic produce of the crop is the processed dried rhizome which varies in color from lemon yellow to dark orange. The use of turmeric dates back to Vedic culture where the crop is reported to be used in both culinary and medicinal preparations. Indian cuisines mainly curry preparations invariably have turmeric as an ingredient which lends its distinctive color and flavor to the dish. The yellow color of the turmeric is due to the presence of bioactive constituent called curcuminoids, comprised mainly of three compounds namely bisdemethoxycurcumin, demethoxycurcumin and curcumin. Apart from curcuminoids, turmeric rhizome contains several volatile organic compounds in the form of essential oil which contributes to earthy flavor of turmeric. The turmeric EO is comprised of monoterpenes and sesquiterpenes compounds namely Ar-turmerone, curlone, β-sesquiphellandrene, α-phellendrene, ar-curcumene, α-terpinolene, β-caryophyllene, etc. Both curcumionoids and essential oil constituents like β- caryophyllene has been reported to have numerous biological activities like antioxidant, anti-inflammatory, anti-cancerous, antihyperlipidemic activity, hypoglycemic, antidiabetic etc., as proved by several *in vitro* and *in vivo* experiments (Dosoky and Setzer, 2018). Thus, increasing the bioactive constituents naturally without genetic modification would result in increasing the value of the crop for industrial exploitation of bioactive metabolites. Among several approaches, use of elicitors (biotic and/or abiotic) has been tried in several food and aromatic crops worldwide. Elicitors are chemical substances of organic or inorganic nature which stimulate the synthesis of secondary metabolites to reinforce the plants defense against any biotic or abiotic stress when introduced at low concentration. These compounds elicit a response in plants which is similar to the defense responses to environmental stimuli or pathogen infection, affecting the plant metabolism and enhancing the synthesis of phytochemicals. These defense responses not only restricted to the synthesis of secondary metabolites but also include the synthesis of defense-related proteins, synthesis of cell wall material to make it more difficult for pathogen entry (Odjakova and Hadjiivanova, 2001; Radman et al., 2003).

Chitosan, a polymer obtained from chitin is the most abundant compound present in the

Earth next to cellulose. Structurally, chitin is composed of repeating unit of *N*-acetylglucosamine monomer. After deacetylation of chitin, chitosan is obtained which is composed of a linear polymer consisting of two sub-units, D-glucosamine and *N*-acetyl-D-glucosamine linked via glycosidic bonds. In agriculture, the application of chitosan has been studied in many crop species like cereals, vegetables, fruits, medicinal, aromatic and ornamental crops. Foliar spray or seed treatment with chitosan was found to increase the chlorophyll and carotenoids content, the photosynthetic rate and induces the plant secondary metabolites synthesis by altering the biosynthetic pathway mainly by inducing the activity of phenylalanine ammonia lyase (PAL) and other enzymes in various crops (Li et al., 2008; Ma et al., 2012; Farouk and Amany, 2012; Jail et al., 2014). Satyabhama et al. (2016) reported that foliar spray of 0.1 % chitosan at monthly interval for seven months increased the production of curcumin in the rhizomes by 100% and curcumin bioaccumulation by 56%.

Salicylic acid (SA) is a phenolic class of plant hormone synthesized by plants in response to various biotic and abiotic factors. Exogenous application of SA known to influence a range of processes like stomatal closure, ion uptake, production of free radicals, induction of antioxidant enzymes and increase in the synthesis of many defense related compounds (Sticher et al., 1997; Khan et al., 2003). Several studies also highlighted the role of SA treatment to increase the secondary metabolite contents in many crop species like Artichoke (Bagherifardet al., 2015), chamomile (Pastirova et al., 2004), peppermint (Figueroa Perezx et al., 2014), coriander, dill and parsley (El-Zaeddi et al., 2017).

Phenylalanine (PHE), an aromatic amino acid involved in the biosynthesis of secondary metabolites is also considered as effective elicitor and biostimulant with positive influence on photosynthesis, plant growth and yield (Hussein et al., 1992; Kowalczyk and Zielony, 2008). Besides, foliar application of amino acid phenylalanine is also found to increase the production of secondary metabolites content in commercially important crop species (Reham et al., 2016; Govindaraju and Arulselvi, 2018; Samani et al., 2019).

The present work was initially planned based on the hypothesis that when plants undergo any biotic and abiotic stress, the stress related cellular signal processing sets in which leads to the reinforcement of plant's protective machinery by increasing the synthesis of certain secondary metabolites in them. By keeping this in our mind, we designed an experiment to study the effect of three chemical elicitors namely chitosan, phenylalanine and salicylic acid on growth, photosynthetic parameters and quality parameters in turmeric. Many times, the experiment conducted at controlled environment would not apply well to field level experiments. Since many studies proved the effectiveness of the above mentioned elicitors in

controlled conditions, we have taken up the experiment in field environment, i.e. rainfed condition at two locations.

Objectives:

Based on this, the objective of the present work was designed as follows:

- **i)** To study the effect of chemical elicitors on growth and physiological parameters
- **ii)** To analyze the biochemical changes induced by chemical elicitors
- **iii)** To study the influence of chemical elicitors on the quality of turmeric rhizomes
- **iv)** To analyze the expression of few genes involved in curcumin biosynthesis using RT-PCR

CHAPTER 2

Background and Outline of the thesis

Plant metabolism is divided mainly into primary and secondary. Primary metabolism mainly consists of synthesis of proteins, carbohydrates and nucleic acids which are the building blocks of cellular system. Secondary metabolism occurs mainly in plants and some lower organisms and it consists of the synthesis of compounds which impart characteristic quality to the plant like colour and fragrance of the flower, pungency of spices, etc. The biosynthetic pathway of secondary metabolites has shared compounds between primary and secondary metabolism. The intermediates from carbohydrates and amino acid metabolism are utilized by specific enzymes to divert its pool to secondary metabolite synthesis.

Secondary metabolites are broadly classified into terpenoids, alkaloids and phenolics based on their chemical structure and route of biosynthesis.

2.1 Terpenoids

Among secondary metabolites, terpenoids constitute a large family of diverse compounds. Steroids, carotenoids, and gibberellic acid are some of its members. They are composed by the most important group of active compounds in plants with over than 23,000 known structures. Many of the terpenoids compounds have pharmacological activity and are used for treating several ailments both in humans and animals. Some of the them are used in the industry as flavours, fragrances and spices. (Styger *et al*, 2010; Gershenzon and Dudareva, 2007). Plant essential oil components are fall in the category of monoterpenes and sesquiterpenes imparting essential oil its characteristics flavor and aroma.

2.2 Alkaloids

As the name suggests, alkaloids are phytocompounds which contains basic nitrogen atoms and are alkaline in nature. In addition to carbon, hydrogen and nitrogen, this group may also contain oxygen, sulfur and rarely other element such as chlorine, bromine and phosphorus. It has a long history of use in medications and later found to have numerous pharmacological effects. Classification of alkaloids is difficult owing to its structural diversity, yet some are classified based on similarity of carbon skeleton. They are biosynthesized from amino acids such as tyrosine and lysine.

2.3 Phenolics

Phenolic compounds from plants are one of largest group of secondary plants constituents synthesized in fruits, vegetables, tea, cocoa, spices and other plants that possess positive health benefits. They have one or more phenolic group in its structure from which the name

"phenolics" was derived. They are generally classified as simple phenolics and polyphenolics. Phenolic compound with single phenol group is termed as simple phenolics. Some of the examples of compounds in this category includes, catechol, resorcinol, hydroquinone, pyrogallol, hydroxyquinol, etc. Phenolics containing carboxylic acid are termed as phenolic acids. Among them, hydroxybenzoic and hydroxycinnamic acids are two main simple phenolic acids found in plants. Phenolic compounds with more than one OH-group in aromatic ring are termed as polyphenols. Based on substitution in carbon atoms, phenolic compounds are divided into subgroups (Al Mamari HH, 2022).

2.4 Elicitors: Introduction

Elicitors are substances of biotic or abiotic origin which induce physiological changes in the plant when introduced at low concentration. Plants respond to these compounds by activating an array of mechanisms, similar to the defense responses to pathogen infections or environmental stimuli, affecting the plant metabolism and enhancing the synthesis of phytochemicals. (Ferrari, 2010). Based on the origin of molecules, elicitors can be classified in to biotic and abiotic elicitors.

Several organic compounds obtained from biological sources are reported to have elicitor activities namely polysaccharides (pectin and cellulose), lipopolysaccharide, chitosan, chitin, alginate, yeast extract, cellulase, glycoprotein, fish protein hydrolysate, lactoferrin, fungal spore, mycelia cell wall and microbial cell wall. Some of the commonly used abiotic elicitors are acetic acid, benzothiadiazole and organic salts namely calcium chloride, copper sulphate and some of the metal ions. Apart from these two categories some of the plant hormones like jasmonic acid, salicylic acid and ethylene are also act as elicitors in many crops (Suvarnalatha et al., 1994; Gorlach et al., 1996; Radman et al., 2003; Poulev et al., 2003; Angelova et al., 2006). Several amino acids are considered a well-known bio-stimulants and bio-elicitors with positive effects on plant growth, yield and to mitigate the stress caused by biotic and abiotic factors. (Kowalczyk and Zielony, 2008).

The elicitors are recognized by the receptors located in the plasma membrane, activates a signal transduction pathway which includes the induction of ion flux channels across the membrane, induction of reactive oxygen species (they act as a downstream signal messenger) and synthesis of signaling hormones like jasmonic acid and salicylic acid These signaling hormones activate the expression of defense related genes such as antioxidant enzymes and enzymes of secondary metabolites synthesis. (Parchman *et al*., 1997; Ellard-Ivey and Douglas 1996; Menke *et al*., 1999; vander Fits and Memelink 2000a). There are numerous reports are available on the effect of amino acids, salicylic acid and chitosan acting as elicitors when

sprayed to plants. The effect includes increase in growth, photosynthesis pigment synthesis, increase in photosynthetic rate, activation of several metabolic enzymes involved in the synthesis of secondary metabolites, etc.

Based on the above literature studies, research work was carried out using three elicitors namely chitosan, phenylalanine and salicylic acid on turmeric crop in two years field experiment. The results were compiled and the final synopsis and dissertation are prepared as following chapters.

Chapter 1: Introduction and Objectives

Chapter 2: Background and Outline of the thesis

Chapter 3: Effect of chemical elicitors on growth, physiology and biochemical parameters of turmeric genotypes

Chapter 4: Foliar spray of chemical elicitors on quality parameters of turmeric genotypes

Chapter 5: Foliar spray of chemical elicitors on expression of *clpks11* gene involved in curcumin biosynthesis in turmeric rhizomes

Chapter 6: General Discussion

Chapter 7: Summary and Conclusion

CHAPTER 3

Effect of chemical elicitors on growth, physiology and biochemical parameters of turmeric genotypes

3.1 Introduction

Plants are sessile. They response to different environmental stimuli including different biotic and abiotic stresses by changing their physiology and biochemical reactions which ultimately leads to protection from those extreme conditions. The same can be mimicked by applying chemical elicitors like chitosan, salicylic acid and phenylalanine to crops to our advantage. The elicitors applied in one stage is found to influence the enzymatic activity in the subsequent stage as proved in the tomato plants (Ortega-Ortiz, 2007). The elicitors are recognized by the receptors located in the plasma membrane which activates a signal transduction pathway which includes the induction of ion flux channels across the membrane, induction of reactive oxygen species (they act as a downstream signal messenger) and synthesis of signaling hormones like Jasmonic acid and Salicylic acid (Odjakova and Hadjiivanova, 2001; Radman et al., 2003). These signaling hormones activate the expression of defense related genes such as antioxidant enzymes and enzymes of secondary metabolites synthesis. (Ellard-Ivey and Douglas, 1996; Menke et al., 1999; vander Fits and Memelink, 2000a).

Among different chemical elicitors, chitosan has been extensively used in different food crops which was reported to increase the photosynthetic rate, elicited the antioxidant machinery systems and also increased the expression of few defense and quality related genes (Li et al., 2008; Ma et al., 2012; Farouk and Amany, 2012; Jail et al., 2014). External application of salicylic acid was reported to be effective in eliciting the biochemical responses in plants (Sticher et al., 1997; Khan et al., 2003). Though amino acids in combination with other chemicals has been tried to boost the growth of the plants, it has also been explored for its elicitors role as they involved in the biosynthesis of diverse secondary metabolites present in many crops (Hussein et al., 1992; Kowalczyk and Zielony, 2008).

Spices plays an important role in Indian cuisines. They add color, flavor and taste to our food preparations. Turmeric is one ubiquitous spice one can find in every kitchen. It is revered as a “golden spice” has been ruling in our country since time immemorial not only as a culinary spice but also as a medicinal plant. It has at least 6000 years of documented history for its use as medicine. It was used in many socio-religious practices in connection with birth, marriage and death. Turmeric is cultivated most extensively in India followed by Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia and Philippines. India is the largest producer,

consumer and exporter of turmeric. (Ravindran et al., 2007).

In the presented work, we have taken three elicitors namely chitosan, phenylalanine and salicylic acid, to study the effect of their foliar spray on turmeric photosynthetic and biochemical machinery in order to find out the influence of these treatment for positive outcome of turmeric growth.

3.2 Materials and Methods

3.2.1. Experimental design

In the year 2017-18, polybag experiment involving two varieties (Pragati and Acc.849), three elicitors and three concentrations of each elicitor were conducted in ICAR-IISR Farm, Kozhikode. The details of the treatments are as follows:

1. Control
2. C1 - chitosan at 100 ppm
3. C2 - chitosan at 200 ppm
4. C3 - chitosan at 500 ppm
5. P1 - phenylalanine at 0.1 mM
6. P2 - phenylalanine at 1 mM
7. P3 - phenylalanine at 10 mM
8. S1 - salicylic acid at 0.01 mM
9. S2 - salicylic acid at 0.1 mM
10. S3 - salicylic acid at 1 mM

In the year 2018-19, a field experiment was conducted at ICAR-IISR Farm, Kozhikode involving four varieties (Pragati, Rajapuri, Acc.849 and Prathibha). The treatment details are same as that of previous year.

In the year 2019-20, single concentration of each elicitor based on the positive result obtained in the previous year was selected and a field experiment was conducted using the same four varieties selected in the previous year at ICAR-IISR experimental Farm, Peruvannamuzhi. The treatment details are as follows:

1. Control
2. CHT- chitosan at 100 ppm
3. PHE – phenylalanine at 1 mM
4. SA - salicylic acid at 1 mM

The experiment was conducted in the open field conditions and was designed in randomized block design with three replications.

3.2.2 Elicitors Treatments

The plants were sprayed with these elicitors by hand sprayer and care was taken to soak both lower and upper surfaces of leaves while spraying. Plants sprayed with 0.02% Tween 20 served as the control. The elicitors were sprayed at rhizome development stage, i.e., 120-150 DAP depending upon the growth duration of the genotypes.

3.2.3 Sample collection and measurements

The measurements on plant height and leaf length and leaf breadth were taken at different time intervals. The leaf gas exchange parameters were measured before the spray and one week after the spray for comparing the effect of treatment. The leaf samples (one sample before the spray and two samples after the spray at one week interval) were collected for the measurement of photosynthetic pigments. Two samplings were done for antioxidant enzyme assays of leaf samples. After the harvest, the yield and dry recovery of the rhizomes were calculated.

3.2.4 Physiological parameters:

3.2.4.1 Measurement of photosynthetic pigments (leaf chlorophyll and carotenoids content)

The chlorophyll content of samples was extracted using 80% acetone as described by Arnon, 1949. The absorbance of the solution was taken at three wavelength and the concentration of chlorophyll a (chl a), chlorophyll b (chl b) and total chlorophyll (Total chl) was calculated using standard formula. The carotenoids content of the sample was measured using the following formula suggested by Yang et al. (1998).

3.2.4.2 Measurement of gas exchange parameters

Gas exchange measurements were taken in the fully expanded third leaf from the top using portable photosynthesis system (LC pro-SD Advanced Photosynthesis Measurement System, England) equipped with the standard leaf chamber (encloses 6 cm^2 of leaf area). The parameters measured were net photosynthetic rate (Pn, μmol m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-2} s^{-1}) and transpiration rate (E, mmolm^{-2} s^{-1}).

3.2.5 Biochemical parameters

3.2.5.1 Measurement of antioxidant enzyme activities

The enzyme extract was prepared by homogenizing the leaves in 3 ml of ice-cold phosphate buffer pH 7.0 containing 1 mM EDTA and 1% Polyvinyl Pyrrolidine (PVP). The content was centrifuged at 7000 rpm for 10 min. and the collected supernatant was used for the estimation of antioxidant enzyme activities and protein content. Catalase (CAT) activity was assayed by following the reduction in the oxidation of hydrogen peroxide by the enzyme at 240 nm as described by Abei (1974) with slight modification. Polyphenol oxidase (PPO) activity was

estimated by the method of Haplin and Lee (1987) with changes in absorbance was monitored at 420 nm. One unit of PPO activity was calculated as the change in absorbance of 0.1 per minute per mg protein of the enzyme extract. Peroxidase (POD) activity was assayed using the method of guiacol oxidation adopted by Putter (1974). One unit of peroxidase is defined as the change in absorbance per minute per mg protein at 436nm. Superoxide dismutase (SOD) activity was assayed using inhibition of photochemical reduction of nitroblue tetrazolium (NBT) using the method suggested by Beyer and Fridovich, 1987. The activity of phenylalanine ammonia lyase (PAL) was assayed by measuring the amount of trans-cinnamic acid formed at 290 nm as suggested by Goldson et al, 2008 and the unit activity was expressed as micromoles of trans-cinnamic acid formed/mL. The protein content of the enzyme extracts was estimated by following the method of Lowry et al. (1951) to calculate the specific activity.

3.2.6 Statistical analysis

Simple analysis of calculating mean, standard deviation, standard error and percent increase was calculated using MS Excel, whereas the multiple means comparisons namely Duncan's Multiple Range Test (DMRT) at 1% and 5% level of significance were done using SAS tool.

3.3 Results

3.3.1 Effect of elicitors on yield and dry recovery (2017-18)

In the polybag experiment in the year 2017-18, only the readings on yield and dry recovery were observed. In the genotype Pragati, C1 treatment showed the highest yield of 0.82 Kg which is followed by C2 and P1 with 0.76 Kgs each. The lowest yield was obtained in S3 (0.42 Kg) and control (0.52 Kg) which was statistically on par with each other. In the genotype, Acc.849, the highest yield was obtained in C2 with average yield of 0.82 Kg. Other treatments which produced higher yield were C3 (0.78 Kg), S3 (0.78 Kg), S1 (0.72 Kg). Chitosan treatment has given higher yield in the genotype Pragati whereas chitosan and salicylic acid has given higher yield in the genotype Acc.849 (Fig 4). The treatments S3 (17.8%), C3 (17.8 %) and P2 (17.7 %) showed significant increase ($P<0.01$) in dry recovery percentage as compared to control in the genotype Acc.849 whereas in the genotype Pragati, significant increase in dry recovery percentage over control was not observed (Fig 1).

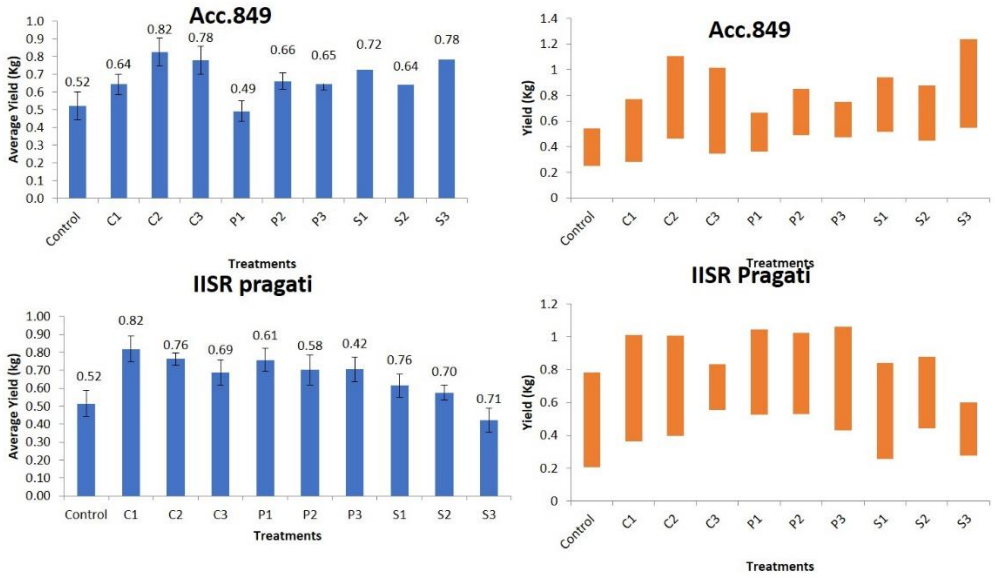


Fig 1. Effect of chemical elicitors on yield of turmeric varieties (2017-18)

3.3.2 Effect of elicitors on growth parameters (2018-19)

In 2018-19 field experiment, in case of Pragati, treatment C2 had given significant higher leaf area (469.24 sq.cm) as compared to control (435. 6 sq.cm). The treatments P1, P2, P3 and S1, S2 and S3 had given significant lower leaf area as compared to control. Almost same trend was observed in case of plant height. Phenylalanine treatment produced significantly higher leaf area in case of the genotype Rajapuri, wherein P1 treatment given the highest leaf area (732.26 sq.cm) as compared to control (529.11 sq.cm). The lowest leaf area values were observed in salicylic acid treated plants. Leaf area was not found significant in the genotype Acc. 849 among the treatments, so as with plant height ($p<0.01$), though treatments C3, P3, S1 and S2 produced significant higher leaf area ($p<0.05$) compared to control.

3.3.2.1 Yield and dry recovery

Treatment related significant changes in yield was observed in the genotype Acc.849 and Prathibha only. In Acc.849, the treatments gave lower yield as compared to control which was in contrast to what we observed in the polybag experiment in the previous year where as in Prathibha almost all treatment has given higher yield than control and only chitosan treatments has resulted in statistically significant higher yield ($P<0.01$). The treatment related changes in yield was not observed in the genotype Rajapuri.

In the genotype Pragati, the treatment showed small variation in dry recovery which was statistically on par ($P<0.05$) with control. In contrast with yield data, the dry recovery percentage of Acc.849 in response to P2 treatment was found to be the highest ($P<0.01$) and all other treatments showed variation at $P<0.05$. The treatments P2 (5 %), C3 (3%) and P3 (3

%) had showed significant increase (P<0.05) in dry recovery percentage as compared to control whereas in other genotypes significant increase in dry recovery percentage over control was not observed.

3.3.3. Effect of elicitors on growth parameters (2019-20)

The plant height and leaf area were recorded over four different time intervals and did not show any statistically significant (P<0.05) differences among control and elicitor treated plants. The yield and dry recovery also did not produce significant differences among treatment.

Though many studies proved the application of elicitors to increase the growth parameters of plants (Reham et al., 2016; Gutiérrez-Gamboa et al., 2018; Satyabhama and Manikandan, 2021), we found only minimal influence of elicitors in our study in both the field experiment. Khan et al., (2002) also reported that foliar application of CHT did not affect plant growth related parameters in maize and soybean. Kadam et al., 2021 also reported that foliar application of chitosan and nano-chitosan-salicylic acid at booting stage of wheat did not influence the growth variables like plant height, tillers and spike length.

The objective of the present experiment was giving elicitor spray to induce the signal transduction pathway which could lead to physiological and biochemical changes in turmeric genotypes and not as growth enhancer. Since the treatments did not produce any negative effect on growth, it could be used safely in the package of practices of turmeric.

3.3.4 Effect of elicitors on photosynthetic pigments (2018-19)

3.3.4.1 Chlorophyll content

Chlorophyll and carotenoids content of leaf samples were taken at two time intervals after the spray. In general, the chlorophyll content of samples collected second week after spray was lower than first week samples. In the genotype Pragati, chl a, chl b and total chl was found to be higher in the treated samples as compared to control. For convenience, only total chlorophyll values are discussed here. Among the treatments, S1 and S2 has given higher total chl content which is 45% higher than control treatment in the first week samples. The treatments S2 (61% increase over control) and C2 (43% increase over control) sustained the chlorophyll retention in the samples collected second week after spray. In the genotype Rajapuri, the treatment P3 gave 29 % increase (P<0.01) in total chlorophyll content over control samples, the next best increase was observed in S3 (16 % increase over control (P<0.05). The treatment S1 has given highest increase (P<0.01) in chlorophyll content (59 %) over control in the second week samples. All other treatments were statistically on par with control. In the genotype Acc.849, the treatment S1 has given 22 % (P<0.01) and 9 % (P<0.05) increase in chlorophyll content in first week and second week samples respectively which was the highest increase in both weeks.

In the genotype Prathibha, S3 and P1 has given the highest increase in chl content of 45% and 43% over control ($P<0.01$) in the first week samples. Overall, salicylic acid treated samples showed higher increase over other treatments. Moreover, this also maintained the improvement of chlorophyll in the second week samples too.

3.3.4.2 Total carotenoids content

In the genotype Pragati, no significant changes were observed in carotenoids content over control in first week samples whereas in the second week samples, significant increase in total carotenoids content was obtained in the treatments P1 (24%) and S2 (12%) over control. In the genotype Rajapuri, the treatments P2, P3 and S3 has given 9%, 15% and 14% increase in total carotenoids content ($P<0.01$) over control in the first week samples. There was significant increase in total carotenoids content in the genotype Acc.849 in the treatments C3 (82 %), P1 (10%), P3 (21%), S1 (16%) and S3 (14%) in first week samples whereas the increase was diminished in the second week samples though treatments C3 (34%) and S1 (60%) has given significantly higher content over control. In the genotype Prathibha, treatments C1 (15%), P1 (23%), S1 (29%) and S3 (15%) showed significant increase in carotenoids content as compared to control in the first week samples and treatments C1 (13%) and S2 (18%) maintained the increase ($P<0.01$) in its content in the second week samples.

3.3.5 Effect of elicitors on photosynthetic pigments (2019-20)

3.3.5.1 Chlorophyll content

The chlorophyll content of control and treated plants were taken at three intervals. First sample was collected before elicitors spray and next two samples were collected after spray at 7-10 days intervals. The readings obtained before spray was taken to measure the inherent variation in the treatment plot and also to compare the changes happened within plot after elicitor spray. In the genotype Pragati, the PHE, CHT and SA treatment showed 35%, 25 % and 17% increase ($P<0.01$) in total chl content in the first samples collected after spray respectively. The chlorophyll content was reduced in elicitor treated plants in the second sample collected after spray as compared to control. In the genotype Rajapuri, in first sampling after spray CHT, PHE and SA treatment increased ($P<0.01$) total chl content to 36%, 24% and 22% respectively. In the genotype Prathibha, the first sample collected after spray, SA, CHT and PHE has given 40%, 33% and 14% increase in total chl content respectively over control. In Acc. 849, the first sampling after spray showed 39%, 16% and 10% increase ($P<0.01$) in total chl content in SA, CHT and PHE treated plants respectively.

3.3.5.2 Total carotenoids content

The total carotenoids content was increased ($P<0.01$) to 33% and 24% in PHE and CHT treated

plants respectively in the first sampling after spray in Pragati. The second sampling after spray showed only moderate increase ($P<0.05$) of 14% and 20% in total carotenoids content as compared to control in the same treatments. In case of Rajapuri, the highest increase ($P<0.01$) of total carotenoids content over control was found in CHT treatment (15%) in the first sampling after spray. In second samples collected after spray, the highest increase ($P<0.01$) of total carotenoids content was found to be in SA treated plants (20%) followed by CHT treatment (10%). Only SA produced significant increase ($p < 0.01$) in total carotenoids content in both first and second sampling in Acc. 849 genotype. In the genotype Prathibha, PHE and CHT produced significant increase ($P<0.01$) of 19% and 10% in samples collected second week after spray.

3.3.6 Effect of elicitors on gas exchange parameters (2018-19)

In the genotype Pragati, in samples collected before elicitors spray, all treatments showed low net photosynthetic rate (Pn) as compared to control. But, after the elicitors spray, the treatments C2, C3 and S3 has produced 25%, 45% and 106% increase ($P<0.01$) in Pn value as compared to control. This was accompanied by increase in transpiration rate (E) and stomatal conductance (gs).

In Acc.849, only S1 treatment has given higher Pn value which was 19% higher than control ($P<0.01$). All other treatments showed increase/decrease in its value ($P<0.05$). But, elicitors spray increased the Pn value of this genotypes. The significant increase ($P<0.01$) was observed in the treatments C3 (37%), S2 (22%), P1 (20%) and S1 (16%) over control. All other treatments were not significantly different from control. In the second week samples, we observed the marginal increase in gs value as compared to the samples before spray though this value was not higher than control samples.

In the genotype Rajapuri, two treatments C1 and C3 showed increased Pn value as compared to control in the samples before the spray. All other treatments showed lower Pn value as compared to control. But, after the elicitors spray, there was significant increase ($P<0.01$) in Pn reading in S3 (44%), C3 (44%), S1 (41%), C2 (23%) treatments as compared to control. The increase was accompanied by increase is E value but no significant increase in gs value was observed in these treatments.

In the genotype Prathibha, only treatments S2 (30%) and P3 (235) showed significant increase over control and few treatments like C2 and P2 showed significant decrease. Whereas the reading taken after the elicitor spray, the treatments C1, P3 and S2 showed 23%, 27% and 16% increase in Pn values respectively as compared to control. The C2 and P2 treatment which showed decrease in Pn reading before spray showed marginal increase in its value after the

elicitors spray. The parameter E showed significant increase over control in all treatments though it did not result in increase in Pn readings in these samples.

3.3.7 Effect of elicitors on gas exchange parameters (2019-20)

The gas exchange parameters were measured at two time intervals; the first reading was taken before the treatment and second reading was taken two week after the spray to compare the effect of treatment on increase or decrease in photosynthetic parameters in the four genotypes. A significant difference in photosynthesis between treatments was observed in all the turmeric genotypes. In Pragati CHT, PHE and SA treatment has produced 47%, 73% and 76% increase ($P<0.01$) in net photosynthetic rate (Pn) respectively. This was accompanied by increase in transpiration rate (E) and stomatal conductance (gs). In the genotype Rajapuri, treatments did not give significant change in Pn and other parameters as compared to control. In Acc. 849, CHT, PHE and SA treatment produced 10%, 29% and 19% increase ($P<0.01$) in Pn. On the other hand, in Prathibha, the CHT and SA treatment increased ($P<0.01$) Pn to 55% and 39% respectively. When comparing the values taken before and after spray, the trend emerged was that only CHT and SA increased the Pn in all the genotypes except Acc. 849.

3.3.8 Effect of elicitors on antioxidant enzyme activities (2017-18)

Polybag experiment conducted in the year 2017-18, four antioxidant enzyme activities like catalase, peroxidase, superoxide dismutase and polyphenol oxidase were measured at two time intervals, i.e. one sample collected in the subsequent week after spray is termed as first week samples and sample collected one week after the spray was termed as second week samples.

In case of CAT activity chitosan was found to be efficient in both Acc.849 and Pragati. In case of POD, the spurt in peroxidase activity was observed in the first week samples as majority of treatments showed significant increase in its activity as compared to control in the genotype Pragati. Phenylalanine treatment was found to increase ($P<0.01$) the peroxidase activity in the genotype Acc.849 in first week samples. Regarding PPO activity, in case of Pragati, no coherent activity changes among treatments were observed in the first week samples, whereas in the second week samples, chitosan treated samples showed highest activity. In the genotype Acc.849, salicylic acid treatments showed increase ($P<0.01$) in PPO activity in first week samples whereas in the second week samples, most of the treatments showed lower activity as compared to control. Concerning SOD activity, in the genotype Pragati, in first week samples, C2 and C3 has given significantly higher activity of SOD as compared to control. In the genotype Acc.849, in first week samples treatments P1, S1 and S2 has given significantly higher ($P<0.01$) SOD activity among treatments.

3.3.9 Effect of elicitors on antioxidant enzyme activities (2018-19)

In the field experiment conducted in the year 2018-19, four antioxidant enzyme activities like catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and polyphenol oxidase (PPO) were measured at two time intervals, i.e. one sample collected the next week after spray is termed as first week samples and sample collected one week after the spray was termed as second week samples. Apart from this, the activity of phenylalanine ammonia lyase (PAL) was measured in the rhizome at two time intervals.

In the genotype Pragati, though elicitors did not produce satisfactory result in the first week samples, they produced the higher activity in second week samples. In the genotype Rajapuri, phenylalanine treatment has given higher activity and they also maintained higher CAT activity in the second week samples. In the genotype Acc.849, chitosan treatments (C3 and C2) has given higher activity. In contrary to Pragati and Acc.849 and Rajapuri, the elicitor treatments increased the CAT activity in the genotype Prathibha in the first week samples. Regarding POD activity, in the genotypes Pragati and Rajapuri, no significant changes ($P<0.01$) in the POD activity was observed as compared to control. In Acc.849, all treatments produced significantly higher activity as compared to control in the first week samples. In the genotype Prathibha, treatments S1, P1 and C3 has given higher POD activity ($P<0.01$) as compared to control in the first week samples.

In case of SOD, in the genotype Pragati, Phenylalanine and salicylic acid has given higher activity than control both in first week and second week samples. In the genotype Rajapuri, almost all treatments showed lower activity than control except C1 and S1 which has given SOD activity on par with control. In the genotype Acc.849, most of the treatments has given lower SOD activity ($P<0.01$). In Prathibha, in first week samples, treatments P2, P3 and C1 has given higher ($P<0.01$) activity as compared to control. Like first week, all salicylic acid treatments have given lower SOD activity in second week samples too.

In case of PPO activity, among genotypes, Prathibha responded well to elicitors treatments as most of the treatment has given significantly higher ($P<0.01$) PPO activity as compared to control. Overall, chitosan treatments have showed higher activity in almost all genotypes among the three elicitors.

In PAL assay, in the genotype Pragati, only salicylic acid treatments have given good activity. The genotype Rajapuri and Acc.849 did not respond well to elicitors treatment with respect to PAL activity. The response of elicitors on PAL activity in the genotype Prathibha is the highest among the genotypes. Among treatments, C3, P2 and S3 has showed the highest ($P<0.01$) PAL activity. Though, other treatments have given higher activity, it is statistically

on par with control.

3.3.10 Effect of elicitors on antioxidant enzyme activities (2019-20)

This initial burst in CAT activity upon elicitor treatments was not observed in our study except in the genotype Prathibha, instead the increased activity ($p < 0.01$) was observed in the samples collected one week after the spray in all the genotypes.

In case of POD activity, both Pragati and Rajapuri did not show any positive influence on its activity whereas Acc.849 and Prathibha showed increase ($p < 0.01$) in its activity in PHE and SA treatment in first sample after spray and both these cultivars did not maintain this increase in activity in second samples.

In SOD activity assay, the genotype Pragati, elicitors treatment did not show any variation in its activity as compared to control in both the week samples. In Rajapuri, in first week samples, PHE treatment has shown higher activity ($P<0.01$) and other treatments did not exert significant influence as compared to control. In second week samples, SA treatment has shown the highest activity ($P<0.01$) and the activity of PHE was on par with control and in chitosan treatment, activity was low compared to other treatments. In Acc.849, SA treatment was given the highest ($P<0.01$) activity in the first week samples followed by PHE. In second week samples, it was the opposite as chitosan treatment have given the highest activity ($P<0.01$) then PHE and SA which also shown higher activity ($P<0.05$) as compared to control. In Prathibha, SOD activity was higher in control in first week samples and in second week, no significant variation in its activity was observed among treatments.

The result of PPO activity showed that, in Pragati and Rajapuri, the treatments did not cause significant positive effect on its activity whereas; in long duration genotypes Acc.849 and Prathibha the treatments not only increased ($p < 0.01$) the activity but also maintained it even one week after the spray.

Regarding activity of PAL, in the genotype Pragati, PHE and SA has given higher activity ($P<0.01$) as compared to control. In the second week samples, it was chitosan which showed the higher activity and activity on SA treatment was on par with control and PHE treatment has given lower activity. In the genotype Rajapuri, chitosan and SA treatment has given the higher activity ($P<0.01$) than control in first week samples, whereas there was no significant difference in second week samples among treatments.

3.4 Discussion

In many field crops, chitosan application was found to produce positive effect on its growth and physiology (Li et al., 2008; Ma et al., 2012; Farouk and Amany 2012) and alleviate the negative effects associated with abiotic stresses like moisture stress, salinity stress, drought and

heavy metal stress. Farouk and Amany (2012) found that foliar application of chitosan to cow pea plants (250 mg/l) have influenced the modification of vascular region of the leaf blade by increased thickness of water storage tissues which could help the plant to cope up with drought stress and to increase the photosynthetic pigments. The stimulating effect of chitosan on plant growth may be attributed to an increase in the availability and uptake of water and essential nutrients through adjusting cell osmotic pressure, and reducing the accumulation of harmful free radicals by increasing antioxidants and enzyme activities (Guan et al.,2009). Numerous studies on chitosan application without any stress treatment produced the significant positive influence on the growth and physiology of different crops like we observed in our study (Boonlertnirun et al., 2008; Salimi et al., 2019; Satyabhama and Manikandan 2021). Likewise, it has been reported that the foliar application of salicylic acid improved the growth and physiology of plants by reducing the ROS mediated damage to photosynthetic pigments. It also improves the activity of chlorophyll synthesizing enzymes (Nazar et al., 2015; Ma et al., 2017). These studies provide corroborative evidence to increased photosynthetic pigments in elicitor treated samples observed in our study. Unlike chitosan and salicylic acid, most of the previous work portrayed phenylalanine as growth stimulant, as being the amino acid, it helps the plant with growth and metabolism. Fewer studies also described the elicitor role of L-phenylalanine application as it is the substrate in phenylpropanoid biosynthesis initiated by the enzyme, phenylalanine ammonia lyase (PAL), the downstream biosynthetic products include phenolics, tannins, coumarins, essential oil, etc. Several studies on application of phenylalanine to elicit defense response of several plants are also available (Patel et al., 2020; Kumar et al., 2020). Foliar application of phenylalanine was also found to increase the photosynthetic pigments in herbal crops as we observed in our study by several previous works. A study by Samani et al., (2019) showed that foliar spray of L- Phenylalanine at 250 ppm significantly increased chl a and chl b content and phenylalanine at 500 ppm increased total carotenoids content and also essential oil content of sage plant. In the same manner, increase in photosynthetic pigment concentration was observed in hyssop plant upon foliar application of L-phenylalanine (Aghaei et al., 2019).

Regarding effect of foliar application of elicitors on photosynthesis, our results were corroborated by previous studies where chitosan spraying in soybean and maize (Khan et al., 2002) and rice (Phothi and Theerakarunwong 2017) and SA on purslane plant (Saheri et al., 2020) and maize (Khan et al., 2003) increased Pn of treated samples as compared to control. Chamnanmanoontham et al., (2015) explained that chitosan treatment induces nucleus and chloroplast genes involved in photosynthesis and antioxidant machinery at chromatin level.

This pattern of increase in net photosynthetic rate and stomatal conductance in the absence of any increase in intercellular CO_2 concentration indicates that the increase is due to enhanced uptake of CO_2 within the leaf that results in improved stomatal conductance, rather than due to having more open stomata (Bittelli et al., 2001). However, correct mechanism behind the increase in photosynthetic rate by certain elicitors like chitosan and salicylic acid is not been elucidated till now. In many plants SA treatment has not only increased the chlorophyll content but also decreased its content which depends on genotypes and concentration of SA used (Cao et al., 2009). We also observed the non-uniformity in the action of SA in different turmeric genotypes we studied. Some study pointed out that prolonged application of salicylic acid resulted in alterations in proteins involved in chloroplast photosynthesis machinery like PSII, the functional activity of which is decreased in prolonged SA treatment (Metodiev et al., 2002; Maslenkova et al., 2009). Coincidently, we used SA in our study only once during rhizome development stage thereby evaded the negative effects of continuous use of SA. The conclusion from our present work is that application of chitosan and salicylic acid produced significant increase in the photosynthetic rate (Pn) of turmeric genotypes irrespective of its different growth nature.

Previous studies have shown that chitosan application causes initial oxidative burst with hydrogen peroxide accumulation in treated plants as well as in cultured cell which stimulates the activity of CAT (Malerba et al., 2011). This initial burst in CAT activity was observed in our polybag experiment (2017-18) in both Pragati and Acc.849. It was not observed in the following two field experiments though the activity was higher in the second week samples. The reason could be that elicitor spray did not cause peroxide burst in these genotypes sans any stress treatment. Satyabhama and Manikandan 2021 found that chitosan application in finger millet increased the POD activity to 9.8 % and other defense enzymes namely polyphenol oxidase, though chitinase did not show any increase. The activities of POD and PPO enzyme have link in lignin biosynthesis which acts as a barrier against biotic and abiotic stresses (Ushamalini et al., 2008; Chandra et al., 2015). The increase in its activity upon elicitor treatment though in genotype specific manner would be beneficial to increases plant innate immunity to protect them against any unforeseeable stresses. Although many papers provided evidence that the antioxidant activity is getting influenced upon elicitor treatment in different crops (Yang et al., 2009, Anusuya and Satyabhama 2016; Satyabhama and Manikandan 2021; Saidi et al., 2021), this is the first study to prove its sustained activity even one week after the spray in turmeric plants. Hundare et al, 2022 reported that foliar spray of 0.5 mM salicylic acid increased the content of photosynthetic pigments in in vitro raised ginger plantlets under salt

stress condition. Moreover, SA treatment was found to increase the antioxidant defense enzyme systems and also reduced the free radicals like hydrogen peroxide and superoxide anion in stressed plants. The study also highlighted that foliar spray of SA was found to be more effective than root feeding as may be the assumption when considering rhizomatous crops like ginger and turmeric. As envisaged in the Fig 1. the elicitors application acts in plants by modulating the endogenous hormones which also includes SA. The leaves and shoots contain higher SA content than root and the exogenous application of SA could increase the endogenous SA level which in turn activate the score of defense pathway (Gharbi et al., 2018). This is the case in most of the biotic and abiotic stress tolerance mechanism in many plants. Though we did not expose the plants to any stresses, the mechanism mentioned above could be related in our present work.

3.5 conclusion

The study found that the elicitors like chitosan, phenylalanine and salicylic acid was found to be efficient in maintaining the higher leaf chlorophyll and carotenoids content in turmeric with slight variation based on genotypes. The elicitors were also found to increase the photosynthetic rate of all the genotypes studied. The present work would be the first in many kinds like including varied genotypes differing in their growth pattern and quality in field level experiment. This gave us the understanding that the influence of elicitors used in the study is not uniform and some genotypes performed better under some elicitors. Although many studies proved the growth stimulating effects of these elicitors in different crops, no study to measure its effect on increasing plant pigments and photosynthetic rate in turmeric was undertaken before as per the available information and hence it is the first to report on the effect of these chemical elicitors on photosynthesis of turmeric plants. The positive result obtained in the present work could be extended by including more locations and more genotypes to estimate the genotype and environmental influence of elicitor activities on turmeric plants to select location and genotype specific elicitors for quality improvement in turmeric.

CHAPTER 4

Foliar spray of chemical elicitors on quality parameters on turmeric genotypes

4.1 Introduction

Turmeric, which is revered as a "golden spice" has been ruling in our country since time immemorial not only as a culinary spice but also as a medicinal plant. It has at least 6000 years of documented history for its use as medicine. It was used in many socio-religious practices in connection with birth, marriage and death. Turmeric is cultivated most extensively in India followed by Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia and Philippines. India is the largest producer, consumer and exporter of turmeric. (Ravindran *et al.,* 2007). Phytochemical evaluation of secondary metabolites of C. *longa* revealed the presence of phenoics and related compounds. Among them curcuminoids are the major diphenyl heptanoid pharmaceutically and commercially. It is a major constituent of *C. longa* rhizomes. Structurally, diphenylheptanoids are composed of two phenyl groups linked through a seven-carbon unit alkane chain. Even though the major chemical principle of turmeric is marketed as curcumin, it consists of the three curcuminoids namely bisdemthoxycurcumin, demethoxycurcumin and curcumin. They are reported to have multi-faceted biological activities including recent research on protection against Alzheimer's disease (Singletary K, 2020, Thakur et al., 2019; Pluta et al., 2022). Apart from curcuminoids, turmeric rhizome contains many terpenoids as components of essential oil with potential pharmaceutical utilization.

Turmeric rhizome is commercially utilized in the form of turmeric powder, turmeric oleoresin and turmeric essential oil. Starch constitutes a major portion in turmeric powder and it has been explored for its non-conventional applications (Santana et al., 2017). Though different cultivars are developed from the same species, the concentration of different chemical constituents vary substantially. Though our country possesses rich cultivar diversity in terms of curcumin content, oleoresin and essential oil, curcumin content is found to be low in some of the highly dominated turmeric growing areas. The agro-climatic conditions, variety and cultivation practices greatly influence curcumin content of the rhizome. (Anandraj et al., 2014). Moreover, nutrient supply, temperature, light conditions and atmospheric CO2 concentrations are some of the environmental factors which influence the levels of secondary metabolites in plants (Fine et al., 2006).

Many studies have established the effectiveness of elicitors like chitosan, carrageenan, sodium alginate, salicylic acid and others in improving the phenolics and terpenoids content in

medicinal and aromatic plants (Shabbir et al., 2017; Goudarzian et al., 2020). Due to its numerous beneficial bioactivities, the need arises to increase the bioactive constituents in turmeric rhizome. Based on the above facts, the study was conducted to test the hypothesis that the foliar application of elicitors like chitosan, salicylic acid and phenylalanine in turmeric would increase the quality profile and bioactive constituents in its rhizome.

4.2 Materials and Methods

4.2.1 Experimental design

Same as that of Chapter 2

4.2.2 Plant Material

Same as that of Chapter 2

Once the above ground vegetative parts (pseudo stem) are dried, rhizomes are harvested, cleaned, cured by boiling them in hot water and dried in the sun for two weeks until the moisture content of the samples were brought down to 10-12 %.

4.3.3 Methodology

The processed, dried rhizomes were pounded in a mortar to crack the rhizome and were pulverized in a mixer and sieved to remove unground material and were used in the analysis of different quality parameters.

4.3.3.1 Estimation of oleoresin

The oleoresin content was measured in a gravimetric method using glass column. The samples were loaded onto the column and were soaked with acetone overnight. In the morning, the content was drained in a pre-measured glass beakers and another 30 mL of acetone was added and were drained in the same container after 1 hour. The acetone extract was vaporized by keeping the beaker over the water bath and the residue was weighed till constant weight was achieved. The percentage of acetone soluble fractions was calculated and are expressed as percentage of oleoresin.

4.3.3.2 Hydrodistillation and GC-MS analysis of volatile constituents

Hydro distillation of essential oil from the dried, powdered rhizomes were done as per the method prescribed in AOAC, 2005. The separation and identification of EO constituents were done in Shimadzu GC/MS fitted with RTX-5 (5 % Phenyl and 95 % dimethyl polysiloxane) column with the dimension of 30 m x 0.25 mm x 0.25 μm. The mass spectra of the components were compared with the standard mass spectral library of NIST/WILEY and identified by similarity search (Adams, 2007).

4.3.3.3 Estimation of Total Curcuminoids content using UV-Visible spectrophotometer

The processed, dried rhizomes were powdered and curcuminoids were extracted with acetone and total curcuminoids (TC) content was calculated following ASTA method 10.2 (ASTA 2014) using UV-Vis Spectrophotometer. The portion of the extracts was filtered using 0.45 μm syringe filter before injecting into HPLC.

4.3.3.4 Estimation of reducing sugars and starch

The content of reducing sugars was done by Nelson-somogyi method (Samogyi, 1952). The starch content in the rhizome was estimated using anthrone reagent as mentioned by Hodge and Hofreiter, 1962.

4.3.3.5 Estimation of total phenolic content

The total polyphenol content of turmeric rhizome was determined using Folin-ciocalteu reagent as per the method described by Singleton and Rossi (1965) with necessary modifications.

4.3.3.6 Estimation of total flavonoid content

Total flavonoid content of turmeric rhizome was determined using aluminum chloride reagents using quercetin as standard in described by Chang et al., 2002 with minor modifications.

4.3.3.7 Estimation of antioxidant activity

Antioxidant activity of methanol extract of turmeric rhizome was done using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays as mentioned by Blois MS, 1958 and Millar et al., 1993 respectively.

4.3.4 Statistical analysis

The data were analysed in SAS software and the treatment mean value (± S.E.) were compared by Duncan's multiple range test (DMRT) ($P<0.01$ and $P<0.05$). Multivariate analysis namely partial least square discriminant analysis (PLS-DA) was conducted on the quality parameters using Metaboanalyst 5.0 online tool.

4.4 Results

4.4.1 Effect of elicitors on quality parameters (2017-18)

4.4.1.1 Curcumin

There was no positive response of elicitors treatment on curcumin content in both the genotypes studied in this polybag experiment. There was no concentration dependent increase or decrease in curcumin content was observed among the treatments.

4.4.1.2 Essential oil

Like curcumin, EO content in the chitosan treated samples showed lower values compared to control in the genotype Pragati. All other treatments showed significant reduction than control ($P<0.01$) except S1 (6.67 %) which showed the highest content on EO among the treatments. In Acc.849, P2 showed the highest EO content (6.2 %) followed by S2 (6.0 %) though they

were not significantly different from Control (5.6 %). All other treatments were on par with control ($P < 0.01$)

4.4.1.3 Oleoresin

Keeping with the trend of other quality parameters, in the genotype Pragati, the treated samples showed significant reduction in the oleoresin content as compared to control. On the other hand, in the genotype Acc.849, treatments S1 (9.9 %), S2 (10.3 %) and S3 (9.8 %) showed significant increase over control (8.5 %) All other treatments showed no significant variation as compared to control.

4.4.1.4 Starch and Sugar

The starch content of the rhizome samples was done and it ranged from 11.8 to 30.0 % in the genotype Pragati and 13.2 % to 31.4 % in the genotype Acc.849. Lot of variation of starch content within treatment chemicals and between treatment and control were found in the genotype analyzed. General significant decline ($P < 0.01$) in starch content was observed among the treatments C3, P1, P3, S1 as compared to control. The treatments C2 (30.0 %) and P2 (19.0 %) showed significant increase as compared to control (17.6 %). If P2 showed increase in starch content in Pragati, it showed the lowest starch content in the genotype Acc.849 (13.2 %) as compared to control (21.0 %). In this genotype, the treatment C1 showed the significant increase ($P > 0.01$) in starch content as compared to control. All other treatments showed no significant variation ($P > 0.01$) as compared to control.

In case of soluble sugar content in the genotype Pragati, only treatment P2 (10.8 %) and S1 (9.5 %) showed significant increase ($P < 0.01$) as compared to control (5.0 %). The treatments C2, P1 and S3 did not show significant change as compared to control. In case of Acc. 849, treatments P3 (7.1 %), C1 (6.8 %), S1 (3.8 %), C2 (4. 0%), P1 (3.5 %), P2 (3.1 %) showed significantly higher soluble sugar content as compared to control.

4.4.2 Effect of elicitors on quality parameters (2018-19)

4.4.2.1 Total curcuminoids and its components

In the genotype Pragati, treatments S2 and S3 has produced 15% and 18% increase in total curcuminoids content ($P<0.01$) over control. It was followed by treatments P1 (13%) and P3 (9%) ($P<0.01$) and C2 (5%) ($P<0.05$) which has given significant increase over control. The curcuminoid components were separated into Bisdemethoxycurcumin (BDMC), Demethoxycurcumin (DMC) and curcumin in HPLC and their concentration is calculated and correlated with total curcuminoids (Table 1).

In the genotype Rajapuri, treatments C3 (12%), C2 (11%), P3 (16%) and S1 (4.6%) has given higher total curcuminoids ($P<0.01$) content. Other treatments were on par with control

(P<0.05). Among the curcuminoid components, only marginal increase in curcumin content was observed (P<0.01) in C3 (9%) and C2 (7%). All other treatments did not show significant difference than control. The same trend was observed in DMC and BDMC also where treatments C2 and C3 has given the highest increase in its content than control (Table 2).

In the genotype Acc.849, treatments S2 (14%), C1 (13%) and P1 (7.5%) has given significant increase (P<0.01) of total curcuminoids content over control. Treatments C3, P2, P3 and S3 has given total curcuminoid content which was on par with control. C2 and S1 has given the lowest content. In case of curcumin content, only the treatment C1 (35%) has given higher (P<0.01) content compared to control (Table 3).

In Prathibha, treatments C1 (25%), P3 (7.5%), C3 (6.7%), C2 (3.6%) and S3 (3%) has shown significant increase (P<0.01) in total curcuminoids content over control. The treatments P1 and S1 has showed significant decrease and other treatments were on par with control. In case of curcumin, the treatments S1 (24%), S3 (7.6 %), C3 (6.4 %), P3 (4.7%), S2 (3.2 %) and C2 (2.6%) has showed significant increase (P<0.01) over control. The increase in BDMC and DMC component of total curcuminoids in response to elicitors treatment was higher in this genotype (Table 4).

4.4.2.2 Essential oil

In the genotype Pragati, the essential oil content of treated samples showed significant increase as compared to control. Among all the treatments, C2 and P3 showed the highest EO content of 5.88 % and 6.0 % respectively which was 10 % and 13 % increase over control. In the genotype Rajapuri, treatments did not increase the EO content as compared to control. It is either statistically on par with control (C1, C2, P3, S1) and lower (C3, P1, P2, S2, S3) than control. In the genotype Acc.849, the treatments P3 (5.33 %) and S1 (5.33 %) has showed highest EO content (P<0.01) which was 9.2% increase over control. The treatments C3 (5.0 %), P1 (5.0 %), P2 (5.17), S2 (5.0 %) and S3 (5.0 %) also showed statistically (P<0.05) significant higher EO content compared to control (4.88 %). In the genotype prathibha, C2 (6.5 %) has given the highest EO content which was 5.5 % higher (P<0.01) as compared to control whereas treatments C1 (6.33 %), P1 (6.17 %), P3 (6.17 %) has given significantly (P<0.05) higher EO as compared to control (6.00 %) (Table 1 & 2 & 3 & 4).

4.4.2.3 Oleoresin

In the genotype Pragati, P1 (12.6 %) showed the highest oleoresin content (P<0.01) which was 9% higher than control (11.52 %). The treatments C2 and P3 also showed significantly higher (P<0.01) oleoresin content as compared to control. In the genotype Rajapuri, C2 (8.59 %) and C3 (8.42 %) showed significantly higher (P<0.05) oleoresin content which was 5% and 3%

higher as compared to control (8.19 %). In Acc.849, treatment S2 (8.6 %) produced the highest oleoresin content which was 10% higher than control (7.83%). Other treatments like C1, C2, S3 also produced higher ($P<0.05$) oleoresin content and other treatments were statistically on par with control. In Prathibha, treatments C2 (15.2 %) and P3 (14.6 %) has given the highest oleoresin content which was 12.2% and 7.7% higher than control (13.6 %). Among other treatments, except C2, others were given lower ($P<0.01$) oleoresin content than control (Table 1 & 2 & 3 & 4).

4.4.2.4 Starch and sugar

In Pragati, Rajapuri and Acc.849 genotypes, treatment related major variation in the starch content ($P<0.01$) was not observed. In prathibha, all treatments showed higher starch content as compared to control. Among treatments, S3 has given 43.7% increase in starch content over control samples (Table 1 & 2 & 3 & 4). The soluble sugar content was found to be higher in P1, C2 and P2 in the genotype Pragati whereas S1 and S2 treatment has given higher content in Rajapuri. In Acc.849, treatments P1 and S2 has given higher sugar content and C1 and S2 has given higher content in the genotype Prathibha.

4.4.2.6 Total phenolics and flavonoids

The treatments related changes in total phenolics and flavonoid content was observed which is varied among genotypes. The response of Pragati towards elicitors treatments concerning these two parameters are minimum as only few treatments especially P3 and S3 has given significant increase in its content over control. In Rajapuri, elicitors treatment did not produce variation in total flavonoid content whereas C2 and C3 produced significant increase ($P<0.01$) in total phenolic content in this genotype and other treatments were statistically on par with control. In the genotype Acc. 849, phenylalanine and salicylic acid treatments has produced significant increase in total flavonoid content and the same trend was observed in total phenolic content also. No increase was observed in chitosan treatment. In Prathibha, a new pattern emerged as phenylalanine and salicylic acid treatments increased the total flavonoid content whereas chitosan treatment has given the highest total phenolic content over control in this genotype (Table 5).

4.4.2.7 Antioxidant activity

Antioxidant activity assay of elicitor treated turmeric rhizome samples were done as ABTS and DPPH free radical quenching ability of rhizome extract. After the analysis, results were represented as % inhibition activity of treatment samples. In 2018-19 field experiment, in the genotype Pragati, treatments S3, S2 and P3 has given higher ($P<0.01$) inhibition of ABTS radical whereas only treatment P1 has given significantly higher inhibition ($P<0.05$) of DPPH

radical. In the genotype Rajapuri, only treatment C3 has given higher ABTS radical inhibition as compared to control whereas treatment C2 has given higher DPPH radical inhibition activity among all treatments. In Acc.849, treatments S2, S3, P1 and P2 has given higher ABTS inhibition, on the other hand S2 and S3 treatment has given lower DPPH radical inhibition activity among treatments. In Prathibha, treatment C1 has given higher DPPH and ABTS inhibition activity among all treatments, whereas all treatments of salicylic acid have given lower percentage of DPPH radical inhibition. In general, salicylic acid treatments have given poor performance in DPPH assay. In some genotypes like Rajapuri, the TFC and TPC content was correlated well with antioxidant activities but in other genotypes, satisfactory correlation could not be found.

4.4.3 Effect of elicitors on quality parameters (2019-20)

4.4.3.1 Curcumin

Among all the genotypes, chitosan treatment showed 16% increase ($P<0.01$) in total curcuminoids content only in Pragati (Table 5). But the HPLC analysis did not show any increase in any of the three curcuminoids components in this genotype. In our study, SA treatment showed significant increase ($P<0.05$) of 9% and 20% ($P<0.01$) of TC content in Pragati and Rajapuri respectively. The same treatment also showed 11% increase ($P<0.01$) in curcumin component in Rajapuri as estimated by HPLC (Table 5). There is no significant increase of TC content in the genotypes Acc.849 and Prathibha in all the treatments (Table 5).

4.4.3.2 Essential oil (EO)

The EO content of turmeric genotypes showed significant variation in response to elicitor treatment (Fig 1). In the genotype Pragati, the treatments P3 (6.0 %), C2 (5.9 %) and S2 (5.6 %) showed significant ($P<0.01$) increase over control (5.3 %) whereas the Rajapuri genotype did not produce any statistically significant increase in the treated plants. In the genotype Acc.849, the treatments P3 (5.3 %) and S1 (5.3 %) produced statistically significant higher EO as compared to control (4.9 %). By comparing the results, variation towards elicitors influence were found among the genotypes studied. Phenylalanine and salicylic acid treatments were effective in the genotype Pragati and Acc. 849 whereas chitosan increased the EO content in Pragati (Table 5).

4.4.3.3 Oleoresin

In the genotype Pragati, PHE and SA have given significant ($P<0.01$) in oleoresin content which was 15% higher over control. Chitosan also showed higher oleoresin content ($P<0.05$) over control. In Rajapuri, only SA treatment has produced significant increase ($P<0.01$) in oleoresin content as compared to control. In Acc.849 and Prathibha, no significant changes in

oleoresin content was observed among treatment (Table 5).

4.4.3.4 Starch and Sugar

In the genotype Pragati, chitosan treatment produced the highest starch content ($P<0.01$) which was 18% higher than control. Other treatments were statistically on par with control. In case of Rajapuri and Acc.849, no significant changes in starch content was observed among treatments. Chitosan treatment has given the highest starch content ($P<0.01$) which was 38% higher than control in the genotype Prathibha. Other treatments were on par with control (Table 5). The highest increase in sugar content was observed in the genotype Rajapuri by the PHE and SA treatments whereas no significant changes were observed in the genotype Prathibha.

4.4.3.6 Total phenolics and flavonoids

In the genotype Pragati, PA treatment has shown the highest total phenolics content ($P<0.01$) which was 24% higher than control. CHA and SA also showed increase ($P<0.05$) in its content which was 12% and 19% higher than control. While flavonoid content was concerned, the same trend was observed as that of phenolics in this genotype. No significant changes in phenolics content was observed among treatment in Rajapuri. In flavonoid content, only chitosan treatment produced significant increase ($P<0.01$) which was 27% higher than control. In Acc.849, no significant changes in phenolics content were observed among treatments. Reduction in flavonoids content in PHE and SA treatment was also observed. In the genotype Prathibha, no significant changes in phenolics and flavonoids content was observed among treatments.

4.4.3.7 Antioxidant activity

In the year 2019-20, only in the genotype Prathibha, treatment SA ($P<0.01$) and CHT and PA ($P<0.05$) has given significant increase in ABTS radical inhibition activity and in case of DPPH, only in the genotype Pragati, treatment PA ($P<0.01$) has given significant higher inhibition as compared to control. All other treatments have given no variation in its activity.

Table 1: Effect of chemical elicitors on quality parameters of the genotype Pragati (2018-19)

Treat-ments	BDMC*	DMC*	Curcumin	TC*	EO*	Oleoresin	Starch
Control	0.77^{D}	0.49^{BC}	2.16^{E}	4.18^{DE}	5.30^{C}	11.52^{CD}	23.61^{ABC}
C1	0.79^{CD}	0.49^{BC}	2.22^{DE}	4.17^{E}	5.50^{BC}	11.47^{D}	22.10^{BC}
C2	0.87^{B}	0.46^{CD}	2.32^{CD}	4.39^{CD}	5.88^{A}	12.20^{B}	23.73^{ABC}
C3	0.77^{D}	0.45^{CD}	2.19^{DE}	4.07^{E}	5.47^{BC}	11.40^{D}	19.91^{C}
P1	0.85^{B}	0.46^{CD}	2.54^{B}	4.76^{AB}	5.00^{D}	12.60^{A}	22.03^{BC}
P2	0.70^{E}	0.43^{CD}	2.13^{E}	4.16^{E}	5.50^{BC}	11.02^{E}	22.96^{BC}

P3	0.84^{BC}	0.54^{B}	2.39^{C}	4.59^{BC}	6.00^{A}	12.03^{B}	23.99^{ABC}
S1	0.46^{F}	0.40^{D}	1.93^{F}	3.81^{F}	5.50^{BC}	10.29^{F}	27.83^{A}
S2	0.92^{A}	0.62^{A}	2.70^{A}	4.82^{A}	5.58^{B}	11.39^{D}	24.64^{AB}
S3	0.97^{A}	0.66^{A}	2.65^{AB}	4.95^{A}	5.50^{BC}	11.70^{C}	25.52^{AB}

*BDMC – Bisdemethoxycurcumin; DMC – Demethoxycurcumin; TC – Total curcuminoids; EO – Essential oil. The data is represented as mean (±SE) from three replicates for each treatment. Means accompanied by the different superscript are significantly different at P<0.01 and means accompanied by at least one common superscript are significantly different at P<0.05.

Table 2: Effect of chemical elicitors on quality parameters of the genotype Rajapuri (2018-19)

Treat-ments	BDMC*	DMC*	Curcumin	TC*	EO*	Oleoresin	Starch
Control	0.05^{B}	0.41^{BC}	1.24^{AB}	1.96^{DE}	5.00^{A}	8.19^{AB}	25.06^{B}
C1	0.03^{D}	0.36^{C}	1.11^{C}	1.89^{E}	5.00^{A}	7.89^{BCD}	25.99^{B}
C2	0.06^{A}	0.48^{B}	1.33^{A}	2.19^{A}	5.00^{A}	8.59^{A}	31.31^{AB}
C3	0.05^{B}	0.60^{A}	1.35^{A}	2.20^{A}	4.77^{AB}	8.42^{A}	31.45^{AB}
P1	0.04^{C}	0.50^{AB}	1.19^{BC}	1.90^{E}	4.62^{B}	7.19^{E}	39.07^{A}
P2	0.04^{C}	0.42^{BC}	1.21^{BC}	1.93^{E}	4.50^{B}	7.61^{CDE}	28.88^{B}
P3	0.04^{C}	0.44^{BC}	1.16^{BC}	2.08^{B}	5.00^{A}	7.62^{CDE}	29.65^{AB}
S1	0.04^{C}	0.43^{BC}	1.19^{BC}	2.05^{BC}	5.00^{A}	7.97^{BC}	30.61^{AB}
S2	0.04^{C}	0.46^{BC}	1.17^{BC}	1.96^{CDE}	4.00^{C}	7.60^{CDE}	28.62^{B}
S3	0.04^{C}	0.41^{BC}	1.17^{BC}	2.04^{BCD}	4.77^{AB}	7.53^{DE}	29.95^{AB}

Table 3: Effect of chemical elicitors on quality parameters of the genotype Acc.849 (2018-19)

Treat-ments	BDMC*	DMC*	Curcumin	TC*	EO*	Oleoresin	Starch
Control	0.05^{CD}	0.54^{DE}	0.34^{B}	1.46^{CD}	4.88^{BC}	7.83^{BC}	30.93
C1	0.07^{A}	0.59^{B}	0.46^{A}	1.65^{A}	4.50^{C}	7.98^{B}	32.33
C2	0.05^{BC}	0.54^{DE}	0.27^{EF}	1.38^{E}	4.77^{BC}	7.98^{B}	29.02
C3	0.06^{B}	0.58^{BC}	0.32^{C}	1.48^{C}	5.00^{AB}	6.43^{D}	33.54
P1	0.04^{E}	0.66^{A}	0.24^{G}	1.57^{B}	5.00^{AB}	7.72^{BC}	32.33
P2	0.04^{DE}	0.57^{BCD}	0.23^{G}	1.42^{DE}	5.17^{AB}	7.27^{C}	32.12
P3	0.06^{BC}	0.52^{EF}	0.28^{D}	1.49^{C}	5.33^{A}	7.29^{C}	30.47
S1	0.04^{E}	0.54^{CDE}	0.26^{F}	1.38^{E}	5.33^{A}	7.29^{C}	33.89
S2	0.04^{E}	0.58^{BCD}	0.28^{DE}	1.67^{A}	5.00^{AB}	8.61^{A}	31.28
S3	0.05^{CD}	0.49^{F}	0.28^{DE}	1.48^{C}	5.00^{AB}	7.97^{B}	33.29

Table 4: Effect of chemical elicitors on quality parameters of the genotype Prathibha (2018-19)

Treat-ments	BDMC*	DMC*	Curcumin	TC*	EO*	Oleoresin	Starch
Control	0.61^{EF}	0.61^{G}	3.44^{E}	5.28^{D}	6.00^{B}	13.57^{B}	21.03^{D}
C1	0.74^{A}	1.04^{A}	3.30^{F}	6.60^{A}	6.33^{AB}	15.22^{A}	22.87^{CD}
C2	0.64^{DE}	0.83^{DE}	3.53^{D}	5.47^{C}	6.50^{A}	13.80^{B}	25.73^{BC}

C3	0.70^{B}	0.93^{BC}	3.66^{B}	5.63^{B}	5.50^{C}	12.72^{C}	28.95^{AB}
P1	0.65^{CD}	0.70^{F}	3.13^{G}	5.10^{E}	6.17^{AB}	12.60^{C}	28.21^{AB}
P2	0.63^{DE}	0.94^{B}	3.13^{G}	5.17^{DE}	5.00^{D}	12.69^{C}	28.48^{AB}
P3	0.71^{B}	0.85^{CD}	3.60^{C}	5.68^{B}	6.17^{AB}	14.62^{A}	26.76^{AB}
S1	0.58^{F}	0.76^{EF}	4.27^{A}	4.74^{F}	5.50^{C}	10.80^{D}	29.09^{AB}
S2	0.58^{F}	0.76^{EF}	3.55^{D}	5.58^{BC}	5.50^{C}	12.94^{C}	26.97^{AB}
S3	0.68^{BC}	0.85^{CD}	3.70^{B}	5.44^{C}	5.33^{CD}	12.79^{C}	30.21^{A}

Table 5: Effect of chemical elicitors on quality parameters of turmeric genotypes (2019-20)

Genotypes	Treat-ments	BDMC*	DMC*	Curcumin	TC*	EO*	Oleoresin	Starch
Pragati	Control	0.62	0.44	1.80	3.19^{B}	4.17^{A}	8.75^{B}	20.30^{B}
	CHT	0.68	0.48	2.05	3.82^{A}	4.83^{A}	9.80^{AB}	24.15^{A}
	PHE	0.71	0.50	1.93	3.59^{A}	4.83^{A}	10.14^{A}	20.79^{B}
	SA	0.64	0.45	1.84	3.54^{AB}	4.17^{A}	10.07^{A}	20.68^{B}
Rajapuri	Control	0.03	0.43	0.25	1.54	4.67^{AB}	7.58	25.06
	CHT	0.04	0.45	0.32	1.60	5.17^{A}	7.73	27.23
	PHE	0.04	0.44	0.27	1.52	4.50^{AB}	7.36	24.41
	SA	0.04	0.48	0.26	1.42	4.33^{B}	6.98	28.83
Acc.849	Control	0.03	0.43	0.25	1.54	4.67^{AB}	7.58	25.06
	CHT	0.04	0.45	0.32	1.60	5.17^{A}	7.73	27.23
	PHE	0.04	0.44	0.27	1.52	4.50^{AB}	7.36	24.41
	SA	0.04	0.48	0.26	1.42	4.33^{B}	6.98	28.83
Prathibha	Control	0.47	0.56	2.66	3.93	4.83	11.58^{A}	21.45^{AB}
	CHT	0.40	0.53	0.44	3.91	4.67	9.85^{A}	29.79^{A}
	PHE	0.46	0.52	2.32	3.59	5.0	9.72^{A}	24.43^{AB}
	SA	0.45	0.44	2.31	4.03	5.0	11.74^{A}	19.28^{B}

BDMC – Bisdemethoxycurcumin; DMC – Demethoxycurcumin; TC – Total curcuminoids; EO – Essential oil. The data is represented as mean (±SE) from three replicates for each treatment. Means accompanied by the different superscript are significantly different at $P<0.01$ and means accompanied by at least one common superscript are significantly different at $P<0.05$

4.4.3 Effect of elicitors on essential oil constituents of turmeric rhizome

The EO constituents analyzed using GC-MS threw more light on the effect of these elicitors on major aroma compounds of turmeric. Based on the results obtained, variations in major volatile constituents comprised of around 70% of relative peak area were studied in detail. The major compounds identified in the EOs of the four genotypes used in the study were ar-turmerone, principal aromatic sesquiterpenoid; curlone (also known as β-turmerone), β-sesquiphellandrene, ar-curcumene, germacrone and zingiberene in the sesquiterpene category. Among monoterpene compounds, α-phellandrene, α-terpinolene, 1,8 cineole and cymene-8-ol occupied significant share in the turmeric EO. By observing differences on the effect of elicitors on monoterpene and sesquiterpene compounds, the volatile constituents were categorized into these two groups and were analyzed statistically.

4.5 Discussion

Turmeric finds application in several traditional medicinal preparations as it is reported to have excellent anti-inflammatory properties. The famous patent-war between India and USA over the use of this crop highlight the importance of turmeric. The extensive research over the decades found out curcuminoids from the rhizomes of turmeric is the reason for its multi-faceted biological activities ranging from anti-inflammatory to anti-cancerous (Singletary K, 2020). Curcumin accumulation in turmeric rhizome starts from 4-5th month after planting and goes on till 8-9th month. This period is crucial and any biotic and abiotic stresses at this stage will affect not only rhizome yield but also the accumulation of curcumin. It is also governed by genotype and environment and its interaction.

Elicitors plays an important role in accumulating bioactive metabolites in different crops as a way to reinforce plant's protective strategy against biotic and abiotic stresses. We explored this knowledge to increase the accumulation of curcumin and other quality compounds in turmeric in the presented study. Moreover, our study is the first detailed results of studying curcuminoids and its components along with other quality parameters in response to three elicitors namely chitosan, phenylalanine and salicylic acid in turmeric.

In our study, influence of chitosan and salicylic acid was found to be higher among genotypes in increasing the curcuminoids and other quality parameters. The increase was maximum and statistically relevant in 2018-19 field experiment as compared to 2019-20, wherein the increase was restricted to few quality parameters like total curcuminoids, oleoresin and starch and genotype specific variation was observed more in 2018-19 field experiment. In 2019-20 many genotypes did not show significant variation in quality parameters. Earlier study by Anusuya et al. (2016) and Satyabhama et al. (2016) reported that chitosan treatment increased total curcuminoids content in turmeric. No reports on the use of phenylalanine and salicylic acid were reported for this crop and ours is the first report in this regard. The main difference between the earlier reported studies and our study is that chitosan was sprayed at three or more times, in which case the chitosan could also act as biofertilizer for improved growth (Hidangmayum et al. 2019) which ultimately could have transcribed into increased curcumin content to the tune of 100% increase. We also observed higher TC content in SA treated plants in 2019-20 field experiment though specific to Pragati and Rajapuri. The increase could be explained by previous researches that SA induces several secondary metabolite biosynthetic pathway genes which would lead to increased accumulation of phenylpropanoids, terpenoids, flavonoids, etc. in cell cultures of different crops (Loc et al. 2016; Rahimi et al. 2014; Zhao et al. 2010). Lan TTP et al. (2019) reported that SA treatment increased the

curcumin accumulation by stimulating the genes involved in its biosynthesis in *Curcuma zedoria* cell cultures. This would be the first report as per our knowledge extends, to show the positive effect of SA treatment in increased curcuminoids accumulation in genotypes dependent manner in turmeric.

While considering increase in EO content on foliar application of chitosan, phenylalanine and salicylic acid, our results were corroborated by the experiment done in other crops (Reham et al., 2016; Alizadeh et al., 2020; Goudarzian et al., 2020; Poorgadhir et al., 2020). The elicitors exert their positive influence on terpenoid metabolism by increasing photosynthetic carbon assimilation products which provides the base compound for the terpenoid biosynthetic pathway (Srivastava et al., 1990; Ahmed et al., 2020). Chitosan application also found to increase the expression of cineole synthase in the roots of sage plants (Vosoughi et al., 2020). Foliar application of chitosan at 0.25 g/L concentration in sage plants enhanced EO content and also increased the concentrations of monoterpene compounds namely limonene, 1,8-cineole, β-thujone and α-humulene (Vosoughi et al., 2018). Apart from volatile constituents, they also reported the increase in the total phenolic and total flavonoid content in chitosan applied plants which emphasize the influence of chitosan on secondary metabolic pathways. Phenylalanine is an aromatic amino acid which is the starting point in the synthesis of many aromatic phenolic compounds including terpenoids in plants. The foliar spray of phenylalanine as growth regulator and also as elicitor to improve the volatile profiling of few crops were available. Garde-Cerdán *et al.*, 2018 studied and found that foliar application of phenylalanine in grapes increased the relative content of volatile compounds such as benzyl alcohol, total benzenoids (aromatic compounds) and total positive compounds whereas total terpenoids and hexen-1-ol were decreased as compared to control.

No reports are available as per our knowledge regarding effect of elicitors foliar spray on oleoresin content in any crop species. Our study is the first to report that chitosan and salicylic acid increased the oleoresin content in Rajapuri and Acc.849 whereas phenylalanine's influence on oleoresin content was prominent in the genotype Pragati and Prathibha. Likewise, reports of elicitors application on rhizome starch content also limited in the research literatures. Sharma et al., 2005 reported that potato plants sprayed with salicylic acid incorporated C^{13} labelled sucrose into starch at higher level than control plants but this accumulation did not necessarily increase the yield of tubers whereas Kowalski et al., 2009 reported that both seed treatment and foliar spray with chitosan increased the yield of potato tubers under subtropical conditions. We found that salicylic acid and phenylalanine has given higher starch content in turmeric genotypes in the year 2018-19 whereas chitosan has given significant increase in

starch content in the genotypes Pragati and Prathibha in the year 2019-20.

In many horticultural crops, the application of chitosan, phenylalanine and salicylic acid was reported to have increased total phenolic and total flavonoid content (Rahman et al., 2018; Housti et al., 2002). In our study also, we found that salicylic acid and phenylalanine was found to be efficient in increasing TFC and TPC in the genotypes Pragati, Acc.849 and Prathibha. Influence of chitosan on enhancing TPC and TFC was minimal and observed only is Rajapuri and Prathibha in the year 2018-19 and in Rajapuri and Acc.849 in the year 2019-20.

3.6 Conclusion

The influence of different elicitors was not uniform among different genotypes. Since this is an open field experiment under natural growing conditions, the concentration dependent decrease or increase in quality constituents were not observed in our study. The response of a plant to any treatment in controlled environment and field trial may not necessarily be same. So, the field experiment under natural growing conditions is need of the hour in such experiments. Based on our field experiment, we conclude that the short duration genotype, Pragati and to some extent Rajapuri, has responded well to elicitors treatment as many parameters showed significant increase compared to control. Findings from the current study indicate that nature based elicitors like chitosan, salicylic acid and phenylalanine could be employed in an environment-friendly manner for sustainable production of high quality turmeric rhizomes and also impart an innate immunity against some of the biotic and abiotic stresses.

CHAPTER 5

Foliar spray of chemical elicitors on expression of *clpks11* gene involved in curcumin biosynthesis in turmeric rhizomes

5.1 Introduction

Curcuminoids, chemically referred to as diphenylheptanoids is a major constituent of turmeric rhizomes. Structurally, diphenylheptanoids are composed of two phenyl groups linked through a seven-carbon unit alkane chain. Curcuminoids are comprised of three components namely bisdemethoxycurcumin (BDMC), demethoxycurcumin (DMC) and curcumin. Mehta and Patel (1980) reported that with increasing maturity, curcumin content of leaf decreased while that of rhizome increased, which indicates that the site of biosynthesis of curcumin is the leaves, from where it gets translocated to rhizome. Being a vegetatively propagated crop, natural variation in curcumin content among different cultivars developed from the same species is limited in turmeric. Though our country possesses rich cultivar diversity in terms of curcumin content, oleoresin and essential oil, areas/zones where turmeric is grown commercially the curcumin content is found to be low. Moreover, climatic influences and soil conditions also affect curcumin content of the rhizome. (Anandraj et al., 2014). Apart from that, nutrient supply, temperature, light conditions and atmospheric CO_2 concentrations are some of the environmental factors which also influence the levels of secondary metabolites in plants (Fine et al., 2006).

Curcuminoids synthesis in turmeric plants follows complex pathway. The work done by Ramirez-Ahumada et al., (2006) thrown light on the involvement of phenylpropanoid pathway in curcumin biosynthesis. The first enzyme involved in the pathway is phenylalanine ammonia lyase (PAL), a branch point enzyme connecting primary and secondary metabolism (Dixon and Paiva, 1995). Some of the other enzymes identified to be involved in curucmin biosynthesis are *p*-coumaroyl shikimate transferase (CST), *p*-coumaroyl quinate transferase (CQT), caffeic acid *O*-methyltransferase (COMT), and caffeoyl-CoA *O*-methyltransferase (CCOMT or CCoAOMT). Two phenylpropanoid unit derived from phenylalanine is condensed with a carbon unit derived from malonyl coA. This reaction is catalyzed by polyketide synthases with detectable curcuminoid synthase activity in turmeric. (Ramirez-Ahumada et al., 2006). In yet another report, Katsuyama et al. (2009) proposed a pathway for curcuminoid biosynthesis in turmeric with two type III PKS.

Sheeja et al., (2015) reported the enzymes involved in curucminoids biosynthetic pathway based on transcriptome sequence analysis of *C. longa and C. aromatica*. They

identified unigenes of Phenylalanine ammonia lyase (PAL), Cinnamate 4-hydroxylase (C4H), 4-Coumarate:coenzyme A ligase (4CL), Coumarate 3-hydroxylase (C3H), Hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT), Caffeoyl CoA O-methyltransferase (COMT), Diketide CoA synthase (DCS), Curcumin synthase 1 (CURS1), Curcumin synthase 2 (CURS2), Curcumin synthase 3 (CURS3) which have involvement in curcumin biosynthesis.

Several polyketide synthases with different specificities towards different substrates were identified which led to the understanding of the variation in compounds structures in the curcumin biosynthesis pathway (Xie *et al.*, 2009; Resmi and Soniya, 2012; Sheeja *et al.*, 2015). Among them one PKS namely *clpks11* was found to have direct correlation with curcumin accumulation in turmeric rhizomes during its developmental stages. (Deepa *et al.*, 2017).

Plants have innate ability to modulate the synthesis of economically important secondary metabolites by sensing the changes in environment, pest and disease attack. The biochemical pathway by which the plant changes its metabolism in response to these changes has been reviewed by many authors (Ganteta and Memelink, 2002) The changes in plant metabolism leads to increase/decrease in synthesis and accumulation of specific metabolites. They may be phenolics in some plants and flavonoids and alkaloids in others which based on the botany and biology of the plant species and also the evolutionary advantages these chemicals confer to that species. The same effect could be brought out in plants by elicitors treatment (Ferrari, 2010; Angelova *et al.*, 2006). They can be considered as vaccines to plants to prepare them for any eventual stress related damages. Several elicitors gave this protection by modulating the expression of genes involved in the synthesis of valuable secondary metabolites like phenolics, terpenoids, alkaloids, etc. (Ref).

Since our objective involved in the study of elicitors spray on quality improvement in turmeric, we analyzed the expression profile of *clpks11*, an important polyketide synthase gene isolated from turmeric which has strong connection with curcuminoids accumulation in the rhizome, in response to elicitors treatment.

5.2 Materials and methods

5.2.1 Experimental design

Same as that of Chapter 2

5.2.2 Plant Material

Same as that of Chapter 2

In the year 2018-19, rhizomes were collected one week after the elicitors spray whereas in the year 2019-20 rhizome samples were collected two time at one week interval after the elicitors

spray. The collected rhizome samples were cleaned from dirt and put it in RNALater and stored in -80^{O}C.

5.2.3 Methodology

5.2.3.1 RNA isolation

Total RNA has been isolated by following the method suggested by Deepa *et al.,* 2014 using acid phenol: chloroform reagent. The isolated RNA was run in agarose gel to check its purity and the concentration were measured using Nanodrop spectrophototmeter. The isolated RNA was given DNAse I treatment by following the protocol of Invitrogen on-column DNase Digestion kit.

5.2.3.2 cDNA synthesis

First-strand cDNA were synthesized from isolated RNA using Thermo fisher RevertAid First strand cDNA synthesis kit. The cDNA integrity were checked by PCR using primers of housekeeping gene Actin.

5.2.3.3 qPCR analysis

Quantitative real time PCR analysis of gene expression was carried out on Rotor-Gene Q (Qiagen) instrument using QuantiFast SYBR Green PCR Kit (Qiagen) by following the protocol mentioned in Sheeja *et al.,* 2015. The Primers used in the analysis is given in table below. Each treatment group had three technical replicates of RNA isolated from three biological replicates and were normalized to the reference gene Ubiquitin.

Table 1: Sequence information of primers used in the study

Clpks11 Fp	TGTCGGAGATCACCCACTTG
*Clpks11*Rp	CGGAGAAGGGAGACCGAGA
Ubiquitin Fp	GCACTCTCGCTGACTACAAC
Ubiquitin Rp	GGCTTGGTGTAGGTCTTCTTC

5.2.3.4 Total curcuminoids (TC) content

The TC content of collected rhizomes (2019-20) were analyzed as per the methods mentioned in 3.3.3.3.

5.2.3.5 Statistical analysis

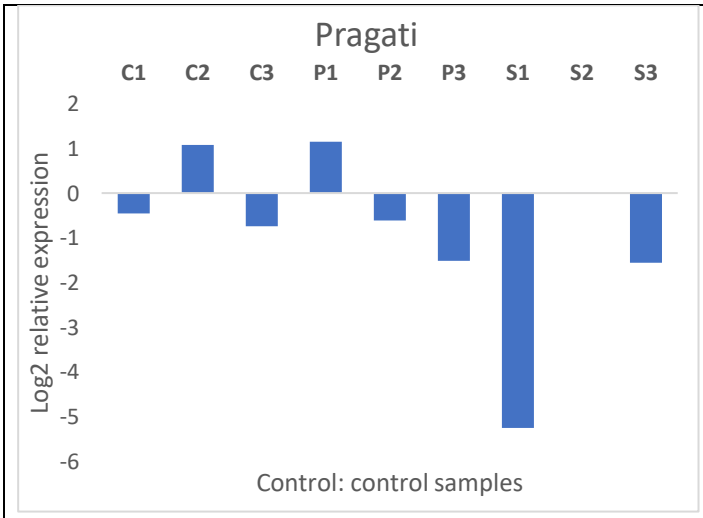
The relative gene expression of *clpks11* was calculated using the $2^{-\Delta\Delta Ct}$ method and transformed to log2 scale (Livak and Schmittgen 2001). The difference in means between treatments in TC content was measured using Duncan Multiple Range Test (DMRT).

5.3 Results

The third objective of the research work involved analyzing expression pattern of major gene involved in curcumin biosynthetic pathway. We had taken *clpks11* gene from previously reported results from our Institute which shows that this gene was found to have direct correlation with its expression and rhizome curcumin content (Deepa *et al.*, 2015). Representative diagram of melt curve of *clpks11* and ubiquitin gene used in the study is given in Fig 1. Likewise, representative diagram of amplification curve of both these gene is given in Fig 2.

5.3.1 Expression analysis of *clpks11* in elicitor treated turmeric rhizomes (2018-19)

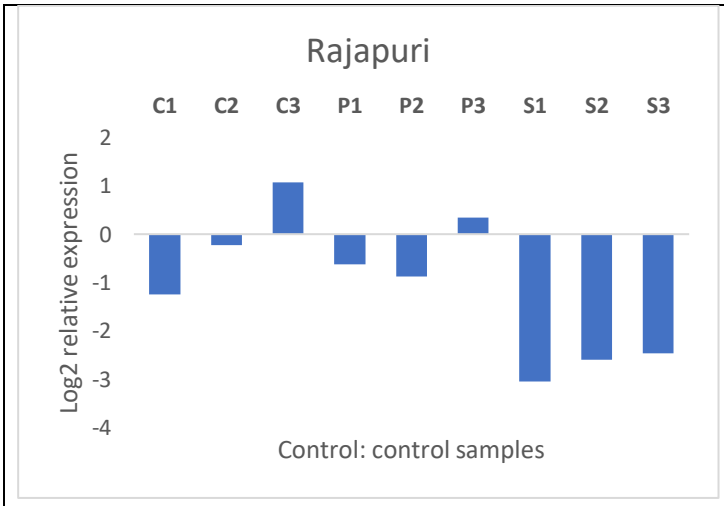
In the genotype Pragati, expression of *clpks11* was higher in the treatments C2 and P1. When comparing curcumin content of harvested rhizome with that of this gene expression, these two treatments has given higher curcumin content compared to control. Though salicylic acid treatment has given higher curcumin content in the rhizome, it was not due to the increased expression of *clpks11*. In Rajapuri, treatments C3 and P3 treatments showed higher expression of *clpks11* as compared to control. Treatment C3 and P3 also has showed significant increase in TC content in this genotype. Remaining treatments showed decrease in expression as compared to control. Though salicylic acid showed higher TC content but not significantly with control (P<0.01). In the genotype Acc.849, all treatments have shown decrease in *clpks11* expression compared to control. Though few treatments like C1, S2 and P1 has showed significant increase in TC content, this was not due to influence of *clpks11* (Fig 5). In Prathibha too, all treatments did not produce increase in *clpks11* expression compared to control (Fig 6).



Treatments	TC Content (%)
Control	4.18^{DE}
C1	4.17^{E}

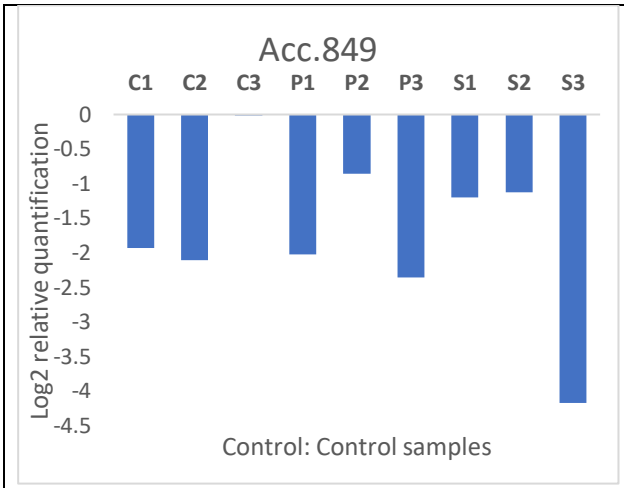
C2	4.39^{CD}
C3	4.07^{E}
P1	4.76^{AB}
P2	4.16^{E}
P3	4.59^{BC}
S1	3.81^{F}
S2	4.82^{A}
S3	4.95^{A}

Fig 3. The relative expression of *clpks11* gene in response to elicitors spray and TC content of harvested rhizome in the genotype Pragati (2018-19)



Treatments	TC Content (%)
Control	1.96^{DE}
C1	1.89^{E}
C2	2.19^{A}
C3	2.20^{A}
P1	1.90^{E}
P2	1.93^{E}
P3	2.08^{B}
S1	2.05^{BC}
S2	1.96^{CDE}
S3	2.04^{BCD}

Fig 4. The relative expression of *clpks11* gene in response to elicitors spray and TC content of harvested rhizome in the genotype Rajapuri (2018-19)

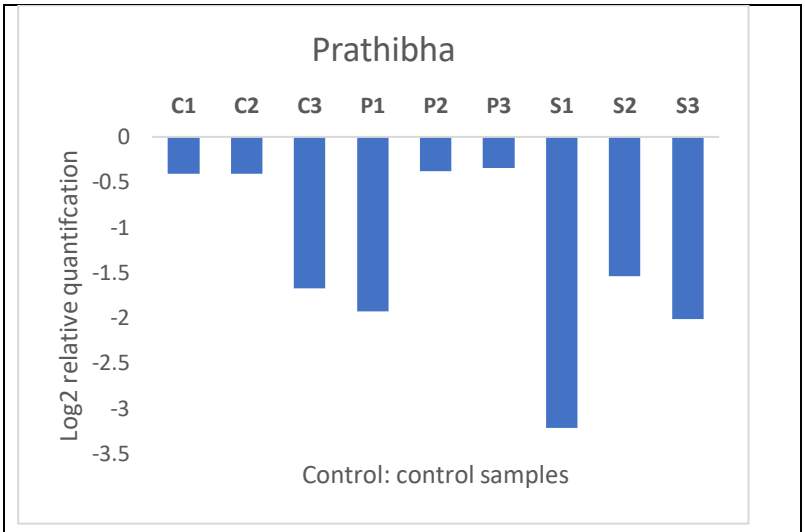


Treatments	TC Content (%)
Control	1.46^{CD}
C1	1.65^{A}
C2	1.38^{E}
C3	1.48^{C}
P1	1.57^{B}
P2	1.42^{DE}
P3	1.49^{C}
S1	1.38^{E}
S2	1.67^{A}
S3	1.48^{C}

Fig 5. The relative expression of *clpks11* gene in response to elicitors spray and TC content of harvested rhizome in the genotype Acc.849 (2018-19)

5.3.2 Expression analysis of *clpks11* in elicitor treated turmeric rhizomes (2019-20)

In 2019-20 field experiment, two set of rhizome samples at one week interval after the elicitor spray were collected. The rhizome samples collected at these two intervals were also dried in an oven and used in the analysis of total curcuminoids for comparison purpose. In case of Pragati, the expression of *clpks11* gene in the sample collected second week after spray showed increased expression as compared to control but the same has not been translated in to increase in curcumin content as no significant difference in its content was observed among treatments (Fig. 7 & Table 2). In the genotype Rajapuri, the treatment showed increase in the expression of *clpks11* gene only in SA where small increment in the expression was found.



Treatments	TC Content (%)
Control	5.28^{D}
C1	6.60^{A}
C2	5.47^{C}
C3	5.63^{B}
P1	5.10^{E}
P2	5.17^{DE}
P3	5.68^{B}
S1	4.74^{F}
S2	5.58^{BC}
S3	5.44^{C}

Fig 6. The relative expression of *clpks11* gene in response to elicitors spray and TC content of harvested rhizome in the genotype Prathibha (2018-19)

The curcumin content showed same pattern as that of expression of *clpks11* gene in this genotype. Salicylic acid treatment showed increase in *clpks11* gene expression in the genotype Acc.849, the first week sample showed maximum increase and second week sample also showed significant increase though less compared to first week sample. But, increase in total curcuminoid content concomitant with increase in gene expression was not found in this genotype (Fig. 7 & Table 2). In the genotype Prathibha, salicylic acid produced significant increase in *clpks11* expression in the first week samples. The total curcuminoid content was higher in the sample collected second week after spray. The analysis showed significant variation among samples and treatments (Fig. 7 & Table 2).

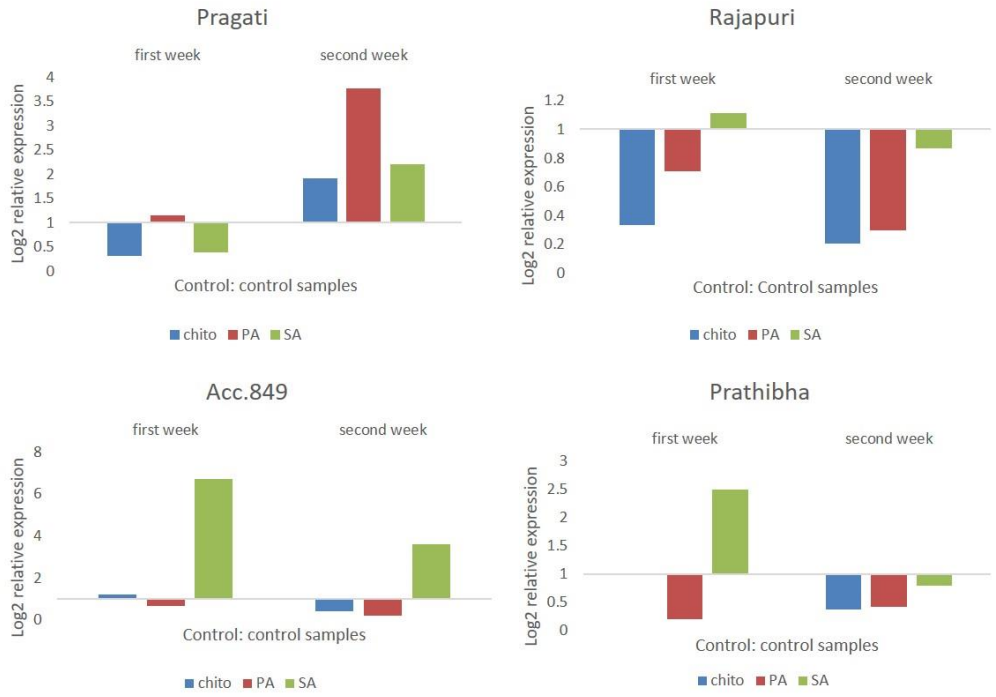


Fig 7. The relative expression of *clpks11* gene in response to elicitors spray in turmeric (2019-20)

Table 2. Total curcuminoids content of rhizome samples after elicitors spray (2019-20)

Genotypes	Treatments	TC content (%)		
		First week	Second week	Matured rhizomes
Pragati	Control	4.63	5.13	3.19
	Chitosan	5.06	4.84	3.82
	PA	6.06	5.11	3.59
	SA	5.02	4.49	3.54
Rajapuri	Control	4.48	2.95	1.64
	Chitosan	3.73	2.63	1.56
	PA	3.84	2.80	1.36
	SA	4.59	2.71	1.90
Acc.849	Control	4.52	2.96	1.54
	Chitosan	3.90	2.85	1.60
	PA	3.82	3.01	1.52
	SA	3.78	2.62	1.42
Prathibha	Control	6.60	6.10	3.93
	Chitosan	6.73	5.39	3.91
	PA	6.19	5.09	3.59
	SA	4.20	6.82	4.03

5.4 Discussion

Curcumin biosynthetic pathway can be roughly divided into upstream and downstream biosynthesis. First section involves the synthesis of early substrates namely p-coumaryl coA, feruloyl CoA, caffoyel CoA, etc. The downstream process mainly involves condensation and formation of curcumin scaffold and its modification thereof. Polyketide synthase enzyme family is an important element in the downstream curcumin biosynthetic pathway. It is involved in the biosynthesis of several phenylpropanoids including phenolics and flavonoids. In turmeric, exploration of curcumin biosynthetic pathway genes leads to identification of several polyketide synthases whose expression level correlated with curcumin accumulation at different stages of rhizome development (Ramirez-Ahumadamdel et al., 2006; Sheeja et al., 2015). In this regard, Deepa et al., 2015 identified a novel polyketide synthase gene, *clpks11* whose expression was highly correlated with rhizome curcumin content at different developmental stages. They also found out that expression of this gene is upregulated in turmeric rhizomes from 60 DAP to 120 DAP when curcumin content of rhizome was maximum. Our experiment also involved the treatment of elicitors at 120-130 DAP after which rhizome samples are collected for gene expression analysis. With limited resources at our disposal, we could analyze the expression of *clpks11* gene involved in the synthesis of curcumin in turmeric rhizomes. We could say that, this is the first report on the activity of curcumin biosynthetic gene expression in response to elicitors treatment in turmeric genotypes. Ayer et al., 2020 reported that genotype and growth stages showed a significant effect on the gene expressions resulting in a significant impact on curcuminoid balance in turmeric rhizome.

Chitosan is reported to influence the expression of several biochemical pathways involving chlorophyll biosynthesis, phenolics, flavonoids and terpenoid biosynthesis in different plant species (Kim et al., 2005; Limpanavech et al., 2008; Kamalipourazad et al., 2016; Vosoughi et al., 2018). Likewise, salicylic acid was also reported to have increased the synthesis of genes involved in both primary and secondary metabolites synthesis in several plant species. Foliar spray of SA was found to increase the expression of PAL gene in saffron (Tajik et al., 2019); *Salvia officinalis* (Ejtahed et al., 2015); *Capsicum annum* (Zunun-Perez et al., 2017), to name a few. Exogenous application of SA also influenced the synthesis of several pharmaceutically important phenolic acid and polyphenolic compounds in many herbs (Ejtahed et al., 2015; Dučaiová et al., 2013; Abbasi et al., 2020). Not many reports on the effect of phenylalanine on the expression of genes involved in secondary metabolites production in any crops is available as per our literature search. Although several reports including our study

showed that chitosan induce the expression of several genes, it is yet to be understand the mechanism of its influence. The influence of these elicitors on gene expression in leaf is direct. Our study analysed the expression profile in rhizome, where curcumin accumulation starts. Our hypothesis was that elicitors like chitosan, phenylalanine and salicylic acid induces the expression of this enzyme in rhizome by modulating the signal transduction pathway which ultimately might have translocated into rhizome and effected the synthesis of secondary metabolites by controlling transcription factors involved in the pathway.

Overall, we prove that elicitors like chitosan, salicylic acid and phenylalanine influences the secondary metabolites production in turmeric rhizome though it is dependent on genotypes and duration of growth. Since, curcumin biosynthetic pathway is very complex with several isoforms of same enzyme involved in the modification of curcumin moiety, we did not find direct correlation with *clpks11* expression with rhizome curcumin content in response to elicitors treatment. Nevertheless, nature-based elicitors like chitosan, phenylalanine and salicylic acid could be used to increase crop productivity and protection. Likewise, they are emerging as cost effective remedy to increase the secondary metabolites production in economically important crop species.

CHAPTER 6

General Discussion

Elicitors are substances of biotic or abiotic origin which modified physiological and biochemical processes in the plant when introduced at low concentration. This includes the activation of different signaling molecules, such as calcium ions, secondary messengers, and protein kinases. This signaling cascade activates genes that are involved in the plant's defense response which leads to the production of defense-related proteins, enzymes, antimicrobial peptides, and phytohormones. In the present work, elicitors such as chitosan (CHT), salicylic acid (SA) and phenylalanine (PA) was selected to assess its effect on biochemical, physiological and quality responses in turmeric. In our study, only minimal effect of elicitors on growth and yield was observed in both the field experiments, though many studies proved the application of elicitors to increase the growth parameters of plants. The more promising effect of elicitors used in the present study was towards photosynthetic pigments like chlorophyll and total carotenoids. To corroborate our results, many studies on chitosan (Boonlertnirun et al., 2008; Salimi et al., 2019; Satyabhama and Manikandan 2021; Ma et al., 2012), salicylic acid (Azeem et al., 2023, Ahmed et al., 2017 and Ma et al., 2017) and phenylalanine (Gutiérrez-Gamboa et al., 2018; Samani et al., 2019 and Aghaei et al., 2019) application produced the significant positive influence on photosynthetic pigments in different crops like the observation made in our study. Foliar application of chitosan and salicylic acid produced showed consistent and significant increase in the photosynthetic rate (Pn) of turmeric genotypes irrespective of its different growth nature. our results were corroborated by previous studies where chitosan spraying in soybean and maize (Khan et al., 2002) and rice (Phothi and Theerakarunwong 2017) and SA on purslane plant (Saheri et al., 2020), maize (Khan et al., 2003) and turmeric (El-sheriff et al., 2023) and PA on soybean (Calzada et al., 2022) and tomato (Almas et al., 2021) was found to increase the photosynthetic rate of treated samples as compared to control. Variations in antioxidant activities in response to elicitors treatments were observed among turmeric genotypes used in the study. The reason for chitosan to induce antioxidant enzyme activity could be due to being positively charged molecule, chitosan has the ability to interacts with negatively charged molecules in the plasma membrane thereby triggering the signal transduction pathway leads to the production of hydrogen peroxide which acts as a signaling molecule to activates the antioxidant enzyme system for neutralizing the free radicals (Pichyangkura and Chadchawan 2015). Specifically, salicylic acid activates a pathway known as the mitogen-activated protein kinase (MAPK) pathway, which ultimately leads to the activation of genes of antioxidant defense enzymes (Zhang and Liu, 2001).

In our study, we observed maximum of 25 % increase in total curcuminoids (TC) content in the genotype Prathibha by chitosan treatment followed by 18% increase in TC content in the genotype Pragati by salicylic acid treatment. Our results were corroborated by reported work by Harish et al., 2022 which showed that spraying of metabolic elicitors like chitosan and salicylic acid at monthly interval till 180 DAP significantly increased (26.95% increase over control) the curcuminoids content. Earlier studies by Anusuya and Satyabhama (2016) and Satyabhama et al. (2016) reported that chitosan treatment increased total curcuminoids content accumulation in turmeric. El-sherif et al., 2022 reported that foliar application of SA (10^{-3} M) increased all three components of curcuminoids in turmeric rhizome as compared to 10^{-4} M concentration. They also correlated the increase with increase in the expression of gens involved in the curcumin biosynthetic pathway (*DKS*, *CURS1*, *CURS2* and *CURS3*). While considering increase in EO content and monoterpene and sesquiterpene constituents on foliar application of chitosan, phenylalanine and salicylic acid, our results were corroborated by the experiment done in other crops (Reham et al., 2016; Ahmed et al., 2016; Alizadeh et al., 2020; Goudarzian et al., 2020; Pirbalouti et al., 2019; Poorgadhir et al., 2020) where increase in EO content in major aromatic crops like Sweet Basil, Hyssop were reported.

Our study also analyzed the effect of elicitors on the expression of *clpks11*, important poly-ketide synthase (PKS) gene involved in the biosynthesis of curcuminoids in turmeric rhizome. In our study in 2018-19, treatments C3 and P3 showed higher expression of clpks11 as compared to control in the genotype Rajapuri. In the year 2019-20, the expression of *clpks11* gene in the sample collected second week after spray showed increased expression as compared to control in the genotype Pragati. In Acc.849, SA treatment showed increase in clpks11 gene expression in the first week sample and second week sample also showed significant increase though less compared to first week sample. But, most of the gene expression when correlated with rhizome TC content, did not correlate well. Only one another study by El-sheriff et al., 2022 reported that foliar spray of salicylic acid (SA) at 10^{-3}M has increased the expression of three type III polyketide synthase genes namely CURS1, CURS2 and CURS3 to 36, 23 and 8 folds respectively in turmeric rhizome.

CHAPTER 7

Summary and Conclusion

Summary

The preliminary work conducted in polybags gave good results in terms of induction in antioxidant enzyme activities and yield. In Pragati, C1 (chitosan @ 100 ppm) has given the highest average yield of 0.82 kg per bag. In the genotype Acc.849, the highest yield of 0.82 kg was obtained in C2 (chitosan @ 200 ppm). In the year 2018-19, field experiment showed salicylic acid was have profound effect on chlorophyll content in Pragati, Rajapuri and Prathibha. The highest increase of 46% was observed in Pragati upon S2 (SA @ 0.1 mM Likewise, SA treatments have given highest influence in increasing total carotenoids content in the genotype Rajapuri, Acc.849 and Prathibha. The highest increase of 32% was observed in S3 (SA @ 1 mM) treatment in Prathibha. Moreover, in Pragati, S3 has given whooping 106 % increase in Pn value followed by 44 % increase in the genotype Rajapuri. In Pragati, P3 has given 13 % increase in EO content, S3 has showed 18% in total curcuminoids (TC) content. Among genotypes, only Prathibha showed positive correlation of yield with many of the quality parameters analyzed. The GC-MS profiling of EO of elicitor treated samples showed that all phenylalanine treatments showed higher content of ar-turmerone and curlone in Pragati. Overall, the performance of Pragati was found to be superior under elicitors treatment. In the following year 2019-20, the field experiment was repeated in Peruvannamuzhi experimental farm under rainfed field condition. In this year, PA treatment has given the highest increase of 36% in chlorophyll content and 34% in total carotenoid content in the genotype Pragati. In the same genotype 47%, 73% and 76% increase photosynthetic rate (Pn) was observed in CHT, PA and SA treatment respectively. In this year, PA treatment has given higher ar-turmerone content in Pragati and Rajapuri genotypes. The elicitor induced expression of *clpks11* gene involved in the curcumin biosynthetic pathway were analyzed in samples of both year field experiment.

Conclusion

Th study provided information that influence of elicitors like chitosan, phenylalanine and salicylic acid was not uniform and performance of some genotypes were found better under some elicitors. Moreover, nature-based elicitors like chitosan, phenylalanine and salicylic acid could be used to increase crop productivity and protection without any harmful effects/residues. They are also emerging as cost effective remedy to increase the secondary metabolites production in economically important crop specie

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References

1. Abbasi BH, Ullah MA, Nadeem M, Tungmunnithum D and Hano C (2020) Exogenous application of salicylic acid and gibberellic acid on biomass accumulation, antioxidant and anti-inflammatory secondary metabolites production in multiple shoot culture of Ajuga integrifolia Buch. Ham. ex D.Don. Industrial Crops and Products 145: 112098.
2. Abei H (1984) Catalyse *in vitro*. Methods enzymol 105:121.
3. Aghaei K, Pirbalouti AG, Mousavi A, Badi HN, Mehnatkesh AM (2019) Effects of foliar spraying of L-phenylalanine and application of bio-fertilizers on growth, yield, and essential oil of hyssop [*Hyssopus officinalis* l. subsp. *Angustifolius* (Bieb.)]. Biocatal Agric Biotechnol 21:101318. https://doi.org/10.1016/j.bcab.2019.
4. Ahmed KBM, Khan MMA, Siddiqui H and Jahan A (2020) Chitosan and its oligosaccharides, a promising option for sustainable crop production- a review. Carbohydrate polymers. 227: 115331. https://doi.org/10.1016/j.carbpol.2019.115331
5. Almas HI, Nisa Z, Anwar S, Kausar A, Farhat F, Munawar M and Khalizadieh R (2021) Exogenous Application of Methionine and Phenylalanine Confers Salinity Tolerance in Tomato by Concerted Regulation of Metabolites and Antioxidants. J Soil Sci Plant Nutr. 21: 3051-3064. https://doi.org/10.1007/s42729-021-00588-9
6. Alizadeh A, Moghaddam M, Asgharzade A and Sourestani MM (2020.)Phytochemical and physiological response of *Satureja hortensis* L. to different irrigation regimes and chitosan

application. Industrial Crops & Products. 158: 112990. https://doi.org/10.1016/j.indcrop.2020.112990

7. Anandaraj M, Prasath D, Kandiannan K, Zachariah TJ, Srinivasan V, Jha AK, Singh BK, Singh AK, Pandey VP, Singh SP, Shoba N, Jana JC, Ravindra Kumar K and Maheswari KU (2014) Genotype by environment interaction effects on yield and curcumin in turmeric (Curcuma longa L.). Industrial Crops and Products. 53: 358- 364. doi:10.1016/j.indcrop.2014.01.005
8. Angelova Z, Georgiev S, Roos W (2006) Elicitation of plants. Biotechnol Biotechnol Equip 20:72-83. https://doi.org/10.1080/13102818.2006.10817345
9. Anusuya S, Sathiyabhama M (2016) Effect of chitosan on growth, yield and curcumin content in turmeric under field condition. Biocatal Agric Biotechnol 6:02–106. https://doi.org/10.1016/j.bcab.2016.03.002.
10. Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1-15. https://doi.org/10.1104/pp.24.1.1.
11. ASTA (2014). Official Analytical Methods, 4thedn. American Spice Trade Association, Washington DC.
12. Ayer DK, Modha K, Parekh V, Patel R, Vadodariya G, Ramtekey V and Bhuriya A (2020) Associating gene expressions with curcuminoid biosynthesis in turmeric. Journal of Genetic Engineering and Biotechnology. 18:83. doi: 10.1186/s43141-020-00101-2.
13. Bagherifard A, Bagheri A, Sabourifard H, Bagherifard G, Najar M (2015) The effect of salicylic acid on some morphological and biochemistry parameters under salt stress in herb artichoke (*Cynara Scolymus L.).* Res J Fish Hydrobiol 10:745-750.
14. Bahl JR, Bansal RP, Garg SN, Gupta MM, Singh V, Goel R and Kumar S (2014). Variation in yield of curcumin and yield and quality of leaf and rhizome essential oils among Indian land races of turmeric Curcuma longa L. Proc. Indian Natl. Sci. Acad. 80: 143-156
15. Beyer WF and Fridovich Y (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Analytical Biochemistry. 161, 559-566.
16. Bittelli MM, Flury G, Campbell S, Nichols EJ (2001) Reduction of transpiration through foliar application of chitosan. Agric For Meteorol 107:167-175. https://doi.org/10.1016/S0168-1923(00)00242-2
17. Blois MS (1958) Antioxidant Determinations by the Use of a Stable Free Radical. Nature 181: 1199-1200.
18. Boonlertnirun S, Boonraung C and Suvanasara R (2008) Application of chitosan in rice production. J Met Mater Miner 18(2): 47-52.

19. Calzada PK, Viciedo OD, Habermann E, Hurtado CA, Gratão LP, Prado DR, Lata-Tenesaca LF, Martinez CA, Ajila Celi GE and Rodríguez JC (2022) Exogenous Application of Amino Acids Mitigates the Deleterious Effects of Salt Stress on Soybean Plants. Agronomy 12:2014.
20. Cao S, Hu Z and Wang H (2009) Effect of salicylic acid on the activities of anti-oxidant enzymes and phenylalanine ammonia-lyase in cucumber fruit in relation to chilling injury. Journal of Horticultural Science & Biotechnology 84: 125–130.
21. Chamnanmanoontham N, Pongprayoon W, Pichayangkura R, Roytrakul S, Chadchawan S (2015) Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast. Plant Growth Regul 75(1):101-114. http://dx.doi.org/10.1007/s10725-014-9935-7.
22. Chandra S, Chakraborty N, Dasgupta A, Sarkar J, Panda K, Acharya K (2015) Chitosan nanoparticles: A positive modulator of innate immune responses in plants. Sci Reports. 5:15195. doi: 10.1038/srep15195.
23. Chang CC, Yang MH, Wen HM and Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis 10(3). https://doi.org/10.38212/2224-6614.2748
24. Deepa K, Sheeja TE, Santhi R, Sasikumar B, Cyriac A, Deepesh PV, Prasath D (2014) A simple and efficient protocol for isolation of high quality functional RNA from different tissues of turmeric (Curcuma longa L.). Physiology and Molecular Biology of Plants 20(2): 263-271. DOI: 10.1007/s12298-013-0218-y
25. Deepa K, Sheeja TE, Babu RO, Srinivasan V, Krishnamurthy KS and Sasikumar B (2017) Highly conserved sequence of *ClPKS11* encodes a novel polyketide synthase involved in curcumin biosynthesis in turmeric (*Curcuma longa* L.). Industrial Crops and Products. 97: 229-241. https://doi.org/10.1016/j.indcrop.2016.12.003.
26. Dixon RA and Paiva NL (1995) Stress-Induced Phenylpropanoid Metabolism. Plant cell 7(7): 1085–1097.
27. Dosoky NS and Setzer WN (2018) Chemical Composition and Biological Activities of Essential Oils of Curcuma Species. Nutrients. 10(9): 196. https://doi.org/10.3390/nu10091196
28. Dučaiová Z, Petruľová V and Repčák M (2013) Salicylic acid regulates secondary metabolites content in leaves of Matricaria chamomilla. Biologia. 68: 904–909. doi: 10.2478/s11756-013-0217-z

29. El-Zaeddi H, Calin-Sanchez A, Nowicka P, Martinez-Tome J, Noguera-Artiaga L, Burlo F, Wojdylo A, Carbonell-Barrachina AA (2017) Preharvest treatments with malic, oxalic, and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley. Food Chemistry. 226:179-186. DOI: 10.1016/j.foodchem.2017.01.067.
30. Ellard-Ivey M and Douglas CJ (1996) Role of Jasmonates in the Elicitor- and Wound-Inducible Expression of Defense Genes in Parsley and Transgenic Tobacco. Plant Physiology. 112(1): 183-192. DOI: 10.1104/pp.112.1.183
31. El-sheriff F, Alkuwayti MA and Khattab S (2022) Foliar Spraying of Salicylic Acid Enhances Growth, Yield, and Curcuminoid Biosynthesis Gene Expression as Well as Curcuminoid Accumulation in *Curcuma longa*. Horticulturae 8:417. https://doi.org/10.3390/horticulturae8050417.
32. Ejtahed RS, Radjabian T and Tafreshi SAH (2015) Expression analysis of phenylalanine Ammonia lyase gene and rosmarinic acid production in Salvia officinalis and Salvia virgata shoots under salicylic acid elicitation. Applied Biochemistry and Biotechnology 176: 1846.
33. Farouk S Amany AR (2012) Improving growth and yield of cowpea by foliar application of chitosan under water stress. Egyptian Journal of Biology. 14:14-26. http://dx.doi.org/10.4314/ejb.v14i1.2.
34. Ferrari S (2010) Biological Elicitors of Plant Secondary Metabolites: Mode of Action and Use in the Production of Nutraceutics. In: Giardi M.T., Rea G., Berra B. (eds) Bio-Farms for Nutraceuticals. Advances in Experimental Medicine and Biology, vol 698. Springer, Boston, MA. https://doi.org/10.1007/978-1-4419-7347-4_12.
35. Ferreira FD, Mossini SAG, Ferreira FMD, Arrotéia CC, Costa CL, Nakamura CV and Machinski Junior M (2013) The inhibitory effects of Curcuma longa L. essential oil and curcumin on Aspergillus flavus Link growth and morphology. The Scientific World Journal, 343804: 6.
36. Figueroa Perez GM, Rozha-Guzman EN, Mercado-Silva E, Loarca-Pina G, Reynoso-Camacho R (2014) Effect of chemical elicitors on peppermint (*Mentha piperita*) plants and their impact on the metabolite profile and antioxidant capacity of resulting infusions. Food Chemistry. 156:273-278. https://doi.org/10.1016/j.foodchem.2014.01.101.
37. Fine PVA, Miller ZJ, Mesones I, Irazuzta S, Appel HM and Stevens MHH (2006) The growth defense trade-off and habitat specialization by plants in Amazonian forest. Ecology. 87**:**S150-S162.

38. Ganteta P and Memelink J (2002) Transcription factors: tools to engineer the production of pharmacologically active plant metabolites. Trends in Pharmacological sciences 12: 563-569.
39. Garde-Cerdán T, Gutiérrez-Gamboa G, López R, Rubio-Bretón P and Pérez-Álvarez EP (2018) Influence of foliar application of phenylalanine and urea at two doses to vineyards on grape volatile composition and amino acids content. Vitis. 57: 137-141. DOI: 10.5073/vitis.2018.57.137-141.
40. Gharbi E, Lutts S, Dailly H and Quinet M (2018) Comparison between the impacts of two different modes of salicylic acid application on tomato (Solanum lycopersicum) responses to salinity. Plant Signal Behav, 13: e1469361.
41. Goldson A, Lam M, Scaman CH, Clemens S and Kermode A (2008) Screening of phenylalanine ammonia lyase in plant tissues, and retention of activity during dehydration. Journal of the Science of Food and Agriculture 88(4): 619-625.
42. Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H, Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell. 8:629-643. DOI: https://doi.org/10.1105/tpc.8.4.629.
43. Goudarzian A, Pirbalouti AG. and Hossaynzadeh, M. 2020. Menthol, Balance of Menthol/Menthone, and Essential Oil Contents of Mentha×Piperita L. under Foliar-Applied Chitosan and Inoculation of Arbuscular Mycorrhizal Fungi. Journal of Essential Oil Bearing Plants. 23(5): 1012-1021. https://doi.org/10.1080/0972060X.2020.1828177
44. Govindaraju S and Arulselvi IP (2018) Effect of cytokinin combined elicitors (L-phenylalanine, salicylic acid and chitosan) on in vitro propagation, secondary metabolites and molecular characterization of medicinal herb – Coleus aromaticus Benth (L). J Saudi Soc Agric Sci. 17:435-444. https://doi.org/10.1016/j.jssas.2016.11.001.
45. Guan YJ, Hu J, Wang X and Shao C (2009) Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. J Zhejiang Univ Sci. 10(6):427-433. doi: 10.1631/jzus.B0820373.
46. Haplin BE and Lee CY (1987) Effect of blanching on enzyme activity and quality changes in green peas. Journal of Food Science. 52:1002-1005.
47. Harish BS, Umesha K, Venugopalan R and Prasad BNM (2022) Photo-selective nets influence physiology, growth, yield and quality of turmeric (*Curcuma longa* L.). Ind Crops Prod. 186:115202.

48. Hidangmayum A, Dwivedi P, Katiyar D and Hemantaranjan A (2019) Application of chitosan on plant responses with special reference to abiotic stress. Physiol Mol Biol Plants. 25: 313-326. https://doi.org/10.1007/s12298-018-0633-1.
49. Hodge JE and Hofreiter BT (1962) Determination of reducing sugars and carbohydrates. In: Whistler, R.L. and Wolfrom, M.L., Eds., Methods in Carbohydrate Chemistry, Academic Press, New York, 380-394.
50. Housti F, Andary C, Gargadennec A and Amssa M (2002). Effect of wounding and salicylic acid on hydroxycinnamoylmalic acids in Thunbergia alata, Plant Physiology and Biochemistry. 40: 761–769.
51. Hundare A, Joshi V and Joshi N (2022) Salicylic acid attenuates salinity-induced growth inhibition in in vitro raised ginger (Zingiber officinale Roscoe) plantlets by regulating ionic balance and antioxidative system. Plant Stress 4: 100070
52. Hussein MS, El-sherbeny SE and Abou Leila BH (1992) Effect of some basic nitrogen compounds on the growth, photosynthetic pigment and alkaloid content in *Datura metal* L. Egypt J Physiol Sci. 16(1-2):141-150.
53. Jail NGD, Luiz C, Neto R and DiPiero RM (2014) High-density chitosan reduces the severity of bacterial spot and activates the defense mechanism of tomato plants. Trop Plant Pathol. 39:434-441. DOI: 10.1590/S1982-56762014000600003
54. Kamalipourazad M, Sharifi M, Maivan HZ, Behmanesh M and Chashmi NA (2016) Induction of aromatic amino acids and phenylpropanoid compounds in Scrophularia striata Boiss. cell culture in response to chitosan-induced oxidative stress. Plant Physiol. Biochem. 107:374–384.
55. Katsuyama Y, Kita T and Horinouchi S. (2009). Identification and characterization of multiple curcumin synthases from the herb *Curcuma longa*. FEBS Lett. 583**:**2799–2803.
56. Khan WM and Prithiviraj B, Smith DL (2002) Effect of foliar application of chitin and chitosan oligosacchardies on photosynthesis of maize and soybean. Photosynthetica. 40(4):621-624.
57. Khan WM, Prithiviraj B and Smith DL (2003) Photosynthetic responses of corn and soybean to foliar application of salicylates. Journal of Plant Physiology. 160:485-492.
58. Kim HJ, Chen F, Wang X, Rajapakse NC (2005) Effect of chitosan on the biological properties of sweet basil (Ocimum basilicum L.). Journal of Agricultural and Food Chemistry. 53: 3696–3701.

59. Kowalczyk K and T Zielony (2008) Effect of Aminoplant and Asahi on yield and quality of lettuce grown on rockwool. Conference of biostimulators in modern agriculture, 7-8 Febuary 2008, Warsaw, Poland.
60. Kumar V, Hatan E, Bar E, Davidovich-Rikanati R, Doron-Faigenboim A, Spitzer- Rimon B, Elad Y, Alkan N, Lewinsohn E and Oren-Shamir M (2020) Phenylalanine increases chrysanthemum flower immunity against *Botrytis cinerea* attack. Plant Jouranl. 104:226-240. https://doi.org/10.1111/tpj.14919.
61. Kurita K (2006) Chitin and chitosan: Functional biopolymers from marine crustaceans. Mar Biotechnol. 8:203-226. https://doi.org/10.1007/s10126-005-0097-5.
62. Lan TTP, Huy ND, Luong NN, Quang HT, Tan TH, Thu LTA, Huy NX and Loc NH (2019). Effect of salicylic acid and yeast extract on curcuminoids biosynthesis gene expression and curcumin accumulation in cells of *Curcuma zedoaria*. Journal of Plant Biotechnology. 46:172-179. https://doi.org/10.5010/JPB.2019.46.3.172.
63. Leela, N. K., Tava, A., Shafi, P. M., Sinu, P. J. and Chempakam, B (2002) Chemical composition of essential oils of turmeric. Acta Pharm., 52: 137-141
64. Li Y, Zhao XM, Xia XY, Luan YS, Du YG and Li FL (2008) Effects of oligochitosan on photosynthetic parameter of *Brassica napus* seedlings under drought stress. Acta Agron Sin. 34:326–329.
65. Limpanavech P, Chaiyasuta S, Vongpromek R, Pichyangkura R, Khunwasim C, Chadchawan S, Lotrakul P, Bunjongrat R, Chaide A and Bangyeekhun T (2008) Chitosan effects of floral production, gene expression and anatomical changes in the Dendrobium orchid. Sci. Hortic. 116(1): 65-72.
66. Loc NH, Giang NT and Huy ND (2016) Effect of salicylic acid on expression level of genes related with isoprenoid pathway in centella (*Centella asiatica* (L.) Urban) cells. 3 Biotech. 6:86. https://doi.org/10.1007/s13205-016-0404-z.
67. Lowry OH, Roscbrough NJ, Farr AL and Randall RL (1951) Protein measurement with Folin phenol reagent. Journal of Biological Chemistry. 193:262.
68. Ma LJ, Li YY, Yu CM, Wang Y, Li XM, Li N, et al. (2012) Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. Protoplasma 249:393-399. https://doi.org/10.1007/s00709-011-0290-5.
69. Ma X, Zheng J, Zhang X, Hu Q and Qian R (2017). Salicylic Acid Alleviates the Adverse Effects of Salt Stress on *Dianthus superbus* (Caryophyllaceae) by Activating Photosynthesis, Protecting Morphological Structure, and Enhancing the Antioxidant System. Front Plant Sci. https://doi.org/10.3389/fpls.2017.00600

70. Malerba M, Cerana R and Crosti P (2011) Defense/stress responses activated by chitosan in sycamore cultured cells. Protoplasma. 249:89-98. DOI: 10.1007/s00709-011-0264-7
71. Maslenkova L, Peeva V, Stojnova Z, Popova L (2009) Salicylic acid-induced changes in photosystem II reactions in barley plants. Biotechnol Biotechnol Equip (23/2009/se special edition/on-line)
72. Mehta KG and Patel RH (1980) Phenotypic stability for yield in nitrogen levels on growth and yield of turmeric (Curcuma longa L.). Karnataka J. Agric. Sci. 9: 483 – 488
73. Menke FL, Champion A, Kijne JW, Memelink JA. A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. EMBO J. 1999;18:4455–4463
74. Metodiev MV, Kicheva MI, Stoinova ZG, Popova LP (2002) Two-dimensional electrophoretic analysis of salicylic acid-inducedchanges in polypeptide pattern of barley leaves. Biol Plant45:585–588
75. Millar NJ, Rice-Evans C, Davies MJ, Gopinathan V and Milner A (1993) A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin Sci (Lond) 84(4):407-12. doi: 10.1042/cs0840407.
76. Nazar R, Umar S and Khan NA (2015) Exogenous salicylic acid improves photosynthesis and growth through increase in ascorbate-glutathione metabolism and S assimilation in mustard under salt stress. Plant Signal Behav. 10:e1003751. doi:10.1080/15592324.2014.1003751.
77. Odjakova M and Hadjiivanova C (2001) Review: The complexity of pathogen defense in plants. Bulgarian Journal of Plant Physiology 27:101-109.
78. Ortega-Ortiz H.Benavides-Mendoza A, Mendoza-Villarreal R, Ramírez-Rodríguez H and De Alba RK (2007) Enzymatic Activity in Tomato Fruits as a Response to Chemical Elicitors. J. Mex. Chem. Soc., 51: 141–144
79. Pastirova A, Repcak M and Eliasova A (2004) Salicylic acid induces chages of coumarin metabolites in *Matricaria chamomilla* L. Plant Science. 167:819-824. https://doi.org/10.1016/j.plantsci.2004.05.027
80. Patel MK, Maurer M, Feygenberg O, Ovadia A, Elad Y, Oren-Shamir M and Alkan N (2020) Phenylalanine: a promising inducer of fruit resistance to postharvest pathogens. Foods. 9(5):646. https://doi.org/10.3390/foods9050646.

81. Pluta R, Furmaga-Jabłońska W, Januszewski S, Czuczwar SJ (2022) Post-Ischemic Brain Neurodegeneration in the Form of Alzheimer's Disease Proteinopathy: Possible Therapeutic Role of Curcumin. *Nutrients*. 14(2):248. https://doi.org/10.3390/nu14020248
82. Phothi R and theerakarunwong CD (2017) Effect of chitosan on physiology, photosynthesis and biomass of rice (Oryza sativa L.) under elevated ozone. Aust. J. Crop Sci. 11: 624-630.
83. Poorgadhir M, Torkashvand AM, Mirjalili SA and Moradi P (2020) Interactions of amino acids (proline and phenylalanine) and biostimulants (salicylic acid and chitosan) on the growth and essential oil components of savory (*Satureja hortensis* L.). Biocatalysis and Agricultural Biotechnology. 30: 101815. https://doi.org/10.1016/j.bcab.2020.101815.
84. Poulev A, O'Neal JM, Logendra S, Pouleva RB, Timeva V, Garvey AS, Gleba D, Jenkins IS, Halpern BT and Kneer R (2003) Elicitation, a new window into plant chemodiversity and phytochemical drug discovery. Journal of Medicinal Chemistry. 46:2542-2547. https://doi.org/10.1021/jm020359t
85. Putter J (1974) Peroxidase. In: Bergmeyer HU (ed), Methods of Enzymatic Analysis, pp.685-690. Verlag Chemie, Weinhan.
86. Radman R, Saex T, Bucke C and Keshavarz T (2003) Elicitation of plants and microbial cell systems. Biotechnol Appl Biochem. 37:91-102.
87. Rahimi S, Devi BSR, Khorolragchaa A, Kim YJ, Kim JH, Jung SK, Yang DC (2014) Effect of salicylic acid and yeast extract on the accumulation of jasmonic acid and sesquiterpenoids in Panax ginseng adventitious roots. Russ J Plant Physiol. 61: 811-817. https://doi.org/10.1134/S1021443714060156.
88. Rahman M, Mukta JA, Sabir AA, Gupta DR, Mohindein M, Hasanuzaman M, Mia MG, Rahman M and Islam MT (2018) Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. Plos One 13: Article e0203769.
89. Ramirez-Ahumada MC, Timmermann BN and Gang DR (2006) Biosynthesis of curcuminoids and gingerols in turmeric (Curcuma longa) and ginger (Zingiber officinale): identification of curcuminoid synthase and hydroxycinnamoyl-CoA thioesterases. Phytochemistry 67(18): 2017-2029.
90. Ravindran PN, Nirmal Babu and Shiva KN (2007) Botany and crop improvement of Turmeric. In: N. Ravindran, K. Nirmal babu, and K. Sivaraman (eds*) Turmeric: the genus Curcuma*, Medicinal and aromatic plants – industrial profiles; v.45. pp 15-70.
91. Reham MS, Khattab ME, Ahmed SS and Kandil MA (2016) Influence of foliar spray with phenylalanine and nickel on growth, yield quality and chemical composition of genoveser basil plant. Afr. J. Agric. Res. 11(16): 1398-1410. DOI: 10.5897/AJAR2015.10699.

92. Resmi MS and Soniya E V(2012) Molecular cloning and differential expressions of two cDNA encoding Type III polyketide synthase in different tissues of Curcuma longa L. Gene 491: 278–283.
93. Saheri F, Barzin G, Pishkar L, Boojar MMA and Babaeekhou L (2020) Foliar spray of salicylic acid induces physiological and biochemical changes in purslame (Portula oleracea L.) under drought stress. Biologia. 75(12):2189-2200. https://doi.org/10.2478/s11756-020-00571-2.
94. Saidi L, Duanis-Assaf D, Galsarker O, Maurer D, Alkan N and Poverenov E (2021) Elicitation of fruit defense response by active edible coatings embedded with phenylalanine to improve quality and storability of avocado fruit. Postharvest Biol Tec. 174:111442. DOI: 10.1016/j.postharvbio.2020.111442
95. Salimi A, OraghiArdebili Z and Salehibakhsh M (2019) Potential benefits of foliar application of chitosan and Zinc in tomato. Iranian Journal of Plant Physiology. 9(2):2703-2708. DOI:10.22034/IJPP.2019.664574
96. Samani MR, Pirbalouti AG, Moattar F and Golparvar AR (2019) L-Phenylalanine and bio-fertilizers interaction effects on growth, yield and chemical compositions and content of essential oil from the sage (Saliva officinalis L.) leaves. Industrial Crops and Products. 137:1-8. https://doi.org/10.1016/j.indcrop.2019.05.019
97. Samogyi M (1952) Notes on sugar determination. Journal of Biological Chemistry 195(1): 19-23.
98. Santana AL, Zabot GL, Osorio-Tobón JF, Johner JCF, Coelho AS, Schmiele M, Steel and Meireles AA (2017) Starch recovery from turmeric wastes using supercritical technology. Journal of Food Engineering 214: 266-276.
99. Sathiyabama M, Bernstein N and Anusuya S (2016) Chitosan elicitation for increased curcumin production andstimulation of defence response in turmeric (*Curcuma longa L.*). Industrial Crops and Products. 89: 87–94. https://doi.org/10.1016/j.indcrop.2016.05.007
100. Sathiyabama M and Manikandan A (2021) Foliar application of chitosan nanoparticle improves yield, mineral content and boost innate immunity in finger millet plants. Carbohydrate Polymers. 258:117691. https://doi.org/10.1016/j.carbpol.2021.117691.
101. Shabbir A, Khan MMA, Sadiq Y, Jaleel H, Ahmad B and Uddin M (2017) Regulation of functional activities and essential oil production in Vetiveria zizanioides L. Nash after γ-irradiated sodium alginate elicitation. Turkish Journal of Biology 41: 661-672.

102. Sharma N, Kaur N and Gupta AK (2005) Effect of salicylic acid on the Carbohydrate Composition and the activities of sucrose metabolizing enzymes in Potato. Indian Journal of Agricultural Biochemistry. 18(1): 43-45.

103. Sheeja TR, Deepa K, Santhi R and Sasikumar B. (2015). Comparative Transcriptome Analysis of Two Species of Curcuma Contrasting in a High-Value Compund Curcumin: Insights into Genetic Basis and Regulation of Biosynthesis. Plant Mol Biol Rep., 33**:** 1825-1836.

104. Singletary K (2020) Turmeric, Potential health benefits. Nutrition Today, 55(1): 45-56 doi: 10.1097/NT.0000000000000392.

105. Singleton VA and Rossi JA (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture 16: 144-158.

106. Srivastava NK, Luthra R and Naqvi R (1990) Relationship of photosynthetic carbon assimilation to essential oil accumulation in developing leaves of Japanese mint. Photosynthetica. 24: 406-411

107. Sticher L, Mauch-Mani B and Metraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol. 35: 235–270.

108. Suvarnalatha G, Rajendran L, Ravishankar GA and Venkataraman LV (1994) Elicitation of anthocyanin production in cell cultures of carrot (*Daucus carota* L.) by using elicitors and abiotic stress. Biotechnol Lett. 16:1275-1280. https://doi.org/10.1007/BF00149631

109. Tajik S, Zarinkumar F, Soltani BM and Nazari M (2019) Induction of phenolic and flavonoid compounds in leaves of saffron (Crocus sativus L.) by salicylic acid. Scientia Horticulturae 257. DOI : 10.1016/j.scienta.2019.108751.

110. Thakur M, Virk R, Sangha PS, Saxena V (2019). The Effects of Turmeric on Alzheimer's Patients. Journal of Food Science and Nutrition Research. 2: 347-353.

111. Ushamalini C, Nakkeeran P and Marimuthu T (2008) Induction of plant defense enzymes in turmeric plants by *Trichoderma viride*. Arch Phytopathol Plant Protec. 41:79-93 https://doi.org/10.1080/03235400600662293

112. Vosoughi N, Gomarian M, Pirbalouri AG, Khaghani SH and Malekpoor F (2020) The effects of water deficit stress and chitosan on cineole synthase gene expression and 1, 8-cineole content in sage (Salvia officinalis). Modern Genetics Journal (MGJ). 14(4): 327-336

113. Vosoughi N, Gomarian M, Pirbalouti AG, Khaghani S and Malekpoor F (2018) Essential oil composition and total phenolic, flavonoid contents and antioxidant activity of sage (*Salvia officinalis* l.) extract under chitosan application and irrigation frequencies. Industrial Crops and Products, 117: 366-374

114. Xie XK, Meehan MJ, Xu W, Dorrestein PC and Tang Y (2009) Acyltransferase mediated polyketide release from a fungal megasynthase. J Am Chem Soc 131(24): 8388–8389

115. Yang CM, Chang KW, Yin MH and Huang HM (1998). Methods for the determination of the chlorophylls and their derivatives. Taiwania. 43(2):116-122.

116. Yang F, Hu J, Li J, Wu X and Qian Y (2009) Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. Plant Growth Regulation. 58:131-136. https://doi.org/10.1007/s10725-009-9361-4.

117. Zhao JL, Zhou LG and Wu JY (2010) Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. Appl Microbiol Biotechnol. 87:137-144. https://doi.org/10.1007/s00253-010-2443-4.

Publications from thesis

1. Sivaranjani. R, Zachariah TJ, Alagupalamuthirsolai M and Thankamani CK (2022). Foliar Spray of Elicitors on Physiology and Quality Responses in Turmeric (*Curcuma longa* L.). Indian Journal of Agricultural Research. 56:567-672. doi:10.18805/IJARe.A-5955.
2. Sivaranjani R and Zachariah TJ (2022). Elicitors induced changes in essential oil constituents of turmeric (Curcuma longa L.) rhizome Journal of Horticultural Sciences.17(1): 237-244.

Research publications in symposium/seminars

1. A poster presentation on “Effect of chemical elicitors on activities of antioxidant enzymes in turmeric (*Curcuma longa L.*) was done at Symposium of spices and Aromatic crops – IX held at SASRD, Nagaland University, Medziphema
2. An Oral presentation titled “Effect of foliar application of chemical elicitors on growth, physiological and biochemical responses of turmeric under rainfed condition” was done in International Plant Physiology Virtual Symposium on “Physiological Intervention for Climate Smart Agriculture” organized by ICAR – Sugarcane Breeding Institute, Coimbatore on 11-12th March, 2021.

Awards received

1. BEST ORAL Paper award for the work "Effect of foliar application of chemical elicitors on growth, physiological and biochemical responses of turmeric under rainfed condition" authored by Sivaranjani. R, John Zachariah T, Alagupalamuthirsolai. M and Thankamani. C.K were presented at International Plant Physiology Virtual Symposium on "Physiological Intervention for Climate Smart Agriculture" organized by ICAR – Sugarcane Breeding Institute, Coimbatore on 11-12th March, 2021.

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